GRAIN SORGHUM RESPONSE TO POSTEMERGENCE APPLICATIONS OF MESOTRIONE AND QUIZALOFOP

by

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B.S., Visayas State University, 1994

AN ABSTRACT OF A DISSERTATION

submitted in partial fulfillment of the requirements for the degree

DOCTOR OF PHILOSOPHY

Department of Agronomy
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Abstract

Growth chamber, greenhouse and field experiments using conventional grain sorghum were conducted to 1) evaluate the differential response of grain sorghum hybrids to POST application of mesotrione at various rates and application timings, and 2) determine the physiology of tolerance of grain sorghum hybrids to mesotrione. Sorghum response ranged from susceptible to tolerant. Mesotrione dose-response studies on four sorghum hybrids revealed that injury symptoms were greatest in Pioneer 85G01 and least in Asgrow Seneca. Mesotrione applied EPOST (early POST) injured sorghum more than when applied at MPOST (mid POST) or LPOST (late POST) timings. Observed injury symptoms were not well correlated with grain yield and were transient, thus injury did not reduce sorghum grain yield. Foliar absorption or translocation of mesotrione in tolerant hybrids did not differ with that of susceptible hybrids but metabolism was more rapid in tolerant than in susceptible hybrids. Initial grain sorghum injury was severe and will likely be a major concern to producers.

Field and growth chambers studies were conducted on herbicide-resistant grain sorghum to 1) determine the effect of quizalofop rates, application timings, and herbicide tank mixes on acetyl-coenzyme A carboxylase (ACCase)-resistant grain sorghum injury and yield, and 2) determine if herbicide metabolism is an additional mechanism that could explain the resistance of ACCase- and acetolactate synthase (ALS)-resistant grain sorghum. Depending on rate, EPOST application caused the greatest injury while the least injury occurred with LPOST application. Crop injury from quizalofop was more prominent at rates higher than the proposed use rate (62 g ha^{-1}) in grain sorghum.

Sorghum grain yield was not affected by quizalofop regardless of rates or application timings. Weed control was greater when quizalofop was applied with other herbicides than when applied alone. Herbicide treatments except those that included 2,4-D caused slight to no sorghum injury. Results of the quizalofop metabolism study do not support the involvement of differential metabolism in the observed response of grain sorghum to quizalofop. Rimsulfuron metabolism by ALS-resistant sorghum is more rapid than the susceptible genotypes, thus explaining the observed rapid recovery of grain sorghum plants from rimsulfuron injury in the field.

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Dedication

This piece of work is dedicated to my parents Tatay Sergs and Nanay Deling. Tay and Nay you have raised us well and I thank God for giving us wonderful parents like you.

Chapter 1 - Differential Response of Grain Sorghum Hybrids to Foliar-Applied Mesotrione

Abstract

The selection of herbicide-resistant weeds in grain sorghum production has prompted researchers to explore alternative herbicides to prevent, delay, and manage herbicide-resistant weed biotypes. Greenhouse and field experiments were conducted to evaluate the differential response of sorghum hybrids to POST application of mesotrione. In a greenhouse experiment, 85 sorghum hybrids were treated with 0, 52, 105, 210 and 315 g ai ha^{-1} mesotrione when plants were at the three- to four-leaf collar stage. Sorghum response ranged from susceptible to tolerant sorghum hybrids. 'Pioneer 84G62', 'Pioneer 85G01', and 'Triumph TR 438' were the most susceptible while 'Dekalb DKS35-70', 'Frontier F222E', and 'Asgrow Seneca' were the most tolerant hybrids. One week after treatment (WAT), the mesotrione rate causing 50% visible injury ranged from 121 to 184 and 64 to 91 g ha^{-1} in the most tolerant and susceptible hybrids, respectively. Mesotrione dose-response studies were conducted under field conditions on four sorghum hybrids. One WAT, injury symptoms were greater (up to 23%) in Pioneer 85G01 than in Asgrow Seneca (< 14%). However, all plants appeared normal by the end of the growing season. In addition, sorghum yields were not reduced by mesotrione treatments as verified by correlation coefficient analysis.

Nomenclature: Mesotrione; sorghum, *Sorghum bicolor* (L.) Moench. SORBI.

Key words: HPPD-inhibiting herbicides, hybrids, visible injury.

Introduction

Grain sorghum is one of the most important cultivated cereal crops in the United States, with an average of 3.3 million hectares harvested per year in the last 5 yr (Anonymous 2007). *Amaranthus* species are one of the most troublesome weeds in grain sorghum (Bridges 1992). Competition studies have shown that when redroot pigweed (*Amaranthus retroflexus* L.) emerged at the 2.6-leaf stage of sorghum, yield loss was 46% (Knezevic et al. 1997). Shipley and Wiese (1969) reported that one *Amaranthus* plant per 30 cm^2 of row in irrigated grain sorghum reduced yield by 48%. In addition, Moore et al. (2004) reported that Palmer amaranth (*Amaranthus palmeri* S.Wats) can increase grain moisture and foreign material in harvested grain sorghum.

Although good cultural practices such as crop rotation are important weed management practices in grain sorghum production, herbicides are the major component of any sorghum weed control program (Brown et al. 2004). Weed control has been achieved with several PRE and POST herbicides such as triazine, chloroacetamides, protoporphyrinogen oxidase (protox)-inhibitors, acetolactate synthase (ALS)-inhibitors, and auxins (Brown et al. 2004; Martin 2004; Rosales-Robles et al. 2005; Shoup et al. 2003; Smith and Scott 2006; Stahlman and Wicks 2000). Atrazine is commonly used in PRE or early POST applications to control several annual broadleaf and grass weeds (Martin 2004). Combinations of chloroacetamide herbicides with atrazine applied PRE control many grass and broadleaf weed seedlings (Smith and Scott 2006). In addition, POST 2,4-D, dicamba, prosulfuron, carfentrazone, or bromoxynil are used to control broadleaf weeds (Rosales-Robles et al. 2005; Smith and Scott 2006).

The selection of herbicide-resistant weeds such as Palmer amaranth, common waterhemp (*Amaranthus rudis* Sauer), redroot pigweed, and Powell amaranth (*Amaranthus powelli* S. Watson) (Anonymous 2008a; Culpepper et al. 2006; Heap 1997) has necessitated management adaptations in grain sorghum production such as tillage and the use of directed herbicides to prevent lower grain yield and quality. Therefore, there is a great need for new herbicide development to delay additional resistance and to help manage herbicide-resistant weed biotypes.

Mesotrione is a selective herbicide that controls many broadleaf and some grass weeds in corn. It disrupts carotenoid biosynthesis by inhibiting the hydroxyphenylpyruvate dioxygenase (HPPD) enzyme, which results in plastoquinone (PQ) synthesis inhibition (Duke et al. 2000; Wichert et al. 1999). PQ is involved in the phosphorylation process and is a cofactor for phytoene desaturase, a necessary enzyme for carotenoid synthesis.

Mesotrione controls troublesome weeds including triazine resistant-species, such as Palmer amaranth, common waterhemp, common lambsquarters (*Chenopodium album* L.), and black nightshade (*Solanum nigrum* L.), as well as weeds that are resistant to ALS inhibitors including *Amaranthus* spp, common cocklebur (*Xanthium strumarium* L.) and annual sowthistle (*Soncus oleraceus* L.) (Sutton et al. 2002). Mesotrione also controls velvetleaf (*Abutilon theophrasti* Medicus), *Ipomoea* spp, prickly sida (*Sida spinosa* L.), and common ragweed (*Ambrosia artemiisifolia* L.) (Armel et al. 2003a; Stephenson et al. 2004).

Currently, mesotrione is labeled for preplant nonincorporated or PRE weed control in grain sorghum. Armel et al. (2003b) reported, however, that without adequate moisture to activate PRE applications of mesotrione, weed control was not sufficient in corn. POST application of mesotrione at 70.5 g ai ha^{-1} demonstrated consistent control of weeds but caused 20% chlorosis in grain sorghum (Horky and Martin 2005). Furthermore, Miller and Regehr (2002) observed that early POST treatments of mesotrione caused severe plant injury, such as 40 to 60% bleaching, but late POST applications caused less injury. Although information is available on the effect of mesotrione application rates and timing on weed control in sorghum, little information is available on sorghum sensitivity among hybrids and grain yield response to POST mesotrione application.

The objectives of this research were to evaluate the differential response of sorghum hybrids to POST-applied mesotrione and to determine if early-season injury symptoms from POST application of mesotrione are predictive of sorghum yields.

Materials and Methods

Greenhouse Study

Eighty-five sorghum hybrids were selected on the basis of differences in maturity, yield potential, and geographical adaptation (Table 1.1). Seeds were sown in row into 54- by 34- by 9.5-cm flats, with six hybrids per flat. The soil mix was a sand:Morrill loam (mesic Typic Arguidolls) soil, 1:1 by volume, with a pH of 7.9 and 1.3% organic matter. Plants were grown under greenhouse conditions of 26/24 C day/night temperatures and 16-h photoperiod with supplemental light intensity of 250 μmol m^{-2} per second photosynthetic photon flux density. Plants were watered as needed and fertilized weekly with a commercial fertilizer[1] solution containing 1.2 g L^{-1} total nitrogen, 0.4 g L^{-1}

phosphorus, and 0.8 g L^{-1} potassium. Before herbicide application, plants were thinned to seven plants per hybrid.

Seedlings of the 85 sorghum hybrids were treated with 0, 52, 105, 210, and 315 g ai ha^{-1} mesotrione at the three- to four-leaf collar stage. Treatments were applied with a bench-type sprayer[2] equipped with an 80015LP[3] tip and calibrated to deliver 187 L ha^{-1} at 138 kPa. The spray mixture included 1% v/v crop oil concentrate (COC)[4]. Control plants were treated with water and 1% v/v COC.

Visible sorghum injury was rated 3, 7, and 14 days after treatment (DAT). Injury ratings were based on a scale of 0 (indicating no injury) to 100% (indicating plant death). Hybrids were classified as tolerant, intermediate, and susceptible if the mean 50% visible injury (ID_{50}) values were significantly higher, the same, or significantly lower than the use rate (105 g ha^{-1}), respectively. The 105 g ha^{-1} rate was used as the benchmark since it is the common use rate of the product. At 14 DAT, sorghum heights were recorded, and then aboveground biomass was determined after plants were dried at 65 C for 5 days.

Field Study

Four sorghum hybrids were selected for this study on the basis of plant response to mesotrione in the greenhouse study. ‘Pioneer 85G01’ (susceptible), ‘Pioneer 84G62’ (susceptible), ‘NC+ 7R83’ (relatively susceptible), and ‘Asgrow Seneca’ (relatively tolerant) sorghum hybrids were planted according to Kansas State University Agricultural Experiment Station and Cooperative Extension Service recommendations (Regehr, 1998) in 2006. Experiments were conducted at Kansas State University research fields at Belleville, Garden City, Hays, Hesston, and Manhattan. Geographic location, soil type, taxonomic class, soil pH, and percentage organic matter were recorded for each soil

(Table 1.2). Plots consisted of four rows spaced at 0.76-m that were 7.5 or 9.1 m long. Weed-free plots were maintained with a PRE application of *S*-metolachlor and atrazine at 1,410 and 1,120 g ai ha^{-1}, and hand hoeing as needed.

Mesotrione was applied at 52, 105, 157, and 210 g ha^{-1} in combination with atrazine at 280 g ha^{-1} when sorghum seedlings were at the three- to five-leaf collar stage. The addition of atrazine to mesotrione treatments is a common, sound weed management practice in corn and sorghum production to increase control of some weed species and lengthen residual control (Stephenson et al. 2004); therefore, inclusion of atrazine in this study facilitates direct applicability to sorghum production. Herbicides were applied by a tractor-mounted sprayer calibrated to deliver 187 L ha^{-1} at 140 or 207 kPa. A nontreated control was included for comparison.

Sorghum plant injury was visually rated 1, 2, 4, and 8 weeks after treatment (WAT) as described in the sorghum hybrid response study. Sorghum grain was mechanically harvested from the middle two rows of each plot. Moisture content and test weight were determined using a grain analyzer[5], and yield was adjusted to 14% moisture.

Experimental Design and Data Analysis

The greenhouse experiment was a randomized complete block design. Treatments were replicated three times, and the experiment was conducted twice. For each hybrid, mesotrione rates that caused 50% visible injury (ID_{50}), biomass reduction (GR_{50}), and height reduction (HR_{50}) were estimated using the nonlinear regression model described by Seefeldt et al. (1995) and Streibig et al. (1993). ID_{50}, GR_{50}, and HR_{50} values were analyzed using ANOVA and means were separated using Fisher's protected LSD at $P \leq 0.05$.

The field experiment was a randomized complete block design, established with a split-plot arrangement of treatments. Main plots were the sorghum hybrids, and subplots were the herbicide rates. Treatments were replicated four times. All data were subjected to ANOVA and means were separated using Fisher's Protected LSD at $P \leq 0.05$. Correlation coefficient analysis on injury vs. yield was performed using PROC CORR procedures of SAS 9.1[6].

Results and Discussion

Greenhouse Study

Foliar applications of mesotrione injured all sorghum hybrids. However, differential responses were observed among sorghum hybrids at all herbicide rates. Mesotrione injury symptoms were characterized by leaf chlorosis and bleaching followed by necrosis and malformation of the tissues. Visible estimates of injury were similar to those observed with other HPPD-inhibiting herbicides (Felix and Doohan 2005; Robinson et al. 2006). These symptoms were more apparent on sorghum 7 DAT than at 3 DAT (data not shown). At 14 DAT, symptoms started to dissipate and new growth appeared normal (data not shown). Crop injury from applications of mesotrione at 315 and 210 g ha^{-1} was more pronounced than at the use rate of 105 g ha^{-1}. There was no interaction between trials for grain sorghum injury, height reduction, and biomass; therefore, data were pooled over trials 1 and 2 for presentation.

Mesotrione injury peaked at 7 DAT, after which plants began to recover. On the basis of visual injury, sorghum response to mesotrione ranged from susceptible to intermediate to tolerant (Table 1.3). ID_{50} values ranged from 121 to 184 g ha^{-1}, 92 to 118 g ha^{-1}, and 64 to 91 g ha^{-1} for the tolerant, intermediate, and susceptible hybrids, respectively. Of the 85 hybrids tested, 23 were classified as susceptible, 45 as intermediate, and 17 as tolerant. Maximum injury from mesotrione application reached 80% for hybrids that were most susceptible (data not shown), but plants were not killed by mesotrione. Pioneer 84G62, Pioneer 85G01, and 'Triumph TR 438' were the most susceptible hybrids, whereas 'Dekalb DKS35-70', 'Frontier F222E', and Asgrow Seneca were the most tolerant hybrids. Differences in sorghum response to mesotrione were not surprising because sorghum hybrids have different progenitors. Differences in genetic background could result in fundamental differences in plant structure, either in the plant cuticle or in transport mechanisms that affect absorption (Bunting et al. 2004). Also, genetic differences might indicate physiological and biochemical differences that could affect translocation and metabolism (Armel et al. 2005). Further studies are required to determine if shoot absorption, translocation, and metabolism of mesotrione vary between tolerant and susceptible sorghum hybrids.

Reductions in plant height and biomass were observed for all hybrids at all herbicide rates tested. HR_{50} for plant height (data not shown) and GR_{50} for biomass (Table 1.3) varied among sorghum hybrids; however, only GR_{50} is presented because correlation coefficient analysis showed that GR_{50} was highly correlated to HR_{50} ($r = 0.78$, $P < 0.0001$). Therefore, GR_{50} alone can be used to estimate HR_{50}. Eight of 17 mesotrione-tolerant hybrids had significantly higher GR_{50} and only 4% (1 of 23) of the mesotrione-susceptible plants had significantly lower GR_{50} than the use rate. Biomass GR_{50} values ranged from 62 to 143 and 105 to 246 g ha^{-1} for susceptible and tolerant hybrids, respectively. These findings were similar to results from research that showed differential response of sweet corn cultivars to mesotrione applied POST (O'Sullivan et al. 2002). Differential response of sweet corn cultivars to POST mesotrione was likely due to differences in metabolism of the foliar-absorbed herbicide. Sweet corn cultivars that were more tolerant to POST applications metabolized mesotrione more rapidly than the sensitive cultivars (Wichert et al. 1999).

Different maturity (early, moderately early, medium, moderately late, and late) was represented among the 85 hybrids tested. However, injury response to mesotrione was not correlated to maturity of the crop (r = -0.12, P = 0.25).

Field Study

Site by hybrid by rate interactions prevented the pooling of data; therefore, data are presented by site, hybrid, and rate for 1 and 2 WAT. Data for sorghum injury at 4 and 8 WAT were not reported because significant differences were not observed among sites, hybrids, rates, or hybrid by rate interactions.

Mesotrione injured all four sorghum hybrids. Injury symptoms in the form of stunting, leaf chlorosis, and necrosis were observed at 1 and 2 WAT, but by 4 WAT these symptoms were only slightly visible. Sorghum injury increased as mesotrione application rate increased. In general, injury symptoms were greatest in Pioneer 85G01 and least in Asgrow Seneca 1 WAT (Table 1.4). NC+ 7R83 and Pioneer 84G62 showed greater injury than Asgrow Seneca but less injury than Pioneer 85G01. At 2 WAT, Asgrow Seneca recovered from mesotrione injury, whereas Pioneer 85G01 continued to show slight injury symptoms. By the end of the growing season, however, all plants appeared normal (data not shown). Symptoms observed in the field were consistent with those observed in the greenhouse study.

Sorghum injury ratings across all four hybrids were greatest at the Hays and Hesston sites and lowest at the Garden City site (Table 1.4). Sorghum injury differences among sites may be due to rainfall received before and after mesotrione application. Five days before mesotrione application, rainfall accumulation was up to 61 and 40 mm at Hays and Hesston, respectively. At Garden City, only a trace of rainfall (0.45 mm) was received. Increased crop injury under high soil moisture has been reported previously (Armel et al. 2003a; Griffin et al. 1994; Wright et al. 1995). At 2 WAT, plants at Hesston continued to have the greatest injury, whereas plants at Garden City showed the least injury. Greater injury at Hesston may be due to greater initial injury of the plants, which led to a longer recovery. Plants at Garden City had less initial injury.

Sorghum grain yields were not significantly reduced despite the severity of mesotrione injury symptoms (Appendix B). Garden City yield data were not included in the analysis because of inconsistencies. Visible mesotrione injury was not well correlated with yield reduction. Correlation coefficient analysis indicated that the injury symptoms observed at 1 WAT and 2 WAT were poorly correlated with sorghum grain yield (Table 1.5). This response suggests that sorghum could tolerate the level of injury observed without yield reductions, which was consistent with previous research (Brown et al. 2004). Among all sites, Hays had the lowest yields in all hybrids (data not shown). This could be attributed to the dry weather conditions, especially during the flowering stage, when precipitation was only 0.25 to 1.5 mm. For the other sites, up to 87 mm precipitation occurred. Yield at Hays was also affected by early frost (Anonymous 2008b).

This study demonstrated that POST application of mesotrione to sorghum hybrids at the seedling stage causes a differential injury response ranging from susceptible to tolerant. However, injury symptoms were not associated with yield reduction. Since sorghum hybrids were able to recover from injury as the growing season progressed, injury symptoms were not good predictors of yield loss.

Several grain sorghum hybrids showed tolerance to POST applications of mesotrione at the seedling stage. However, further research is required to verify the extent of crop injury by POST mesotrione applied at different plant stages to ensure that mesotrione is not applied at highly sensitive stages of the crop. Mesotrione could improve broadleaf weed control in grain sorghum production systems by providing growers with an effective POST herbicide option, especially for control of triazine, ALS-, protox-, and EPSPS-resistant weeds.

Sources of Materials

[1]Miracle-gro soluble fertilizer, Scotts Miraacle-Gro products Inc, 1411 Scottslawn Road, Marysville, OH 43041.

[2]Research track sprayer, De Vries Manufacturing, RR 1, Box 184, Hollandale, MN 56045.

[3]TeeJet, Spraying Systems Co., Wheaton, IL 60189-7900.

[4]Prime Oil, Terra International Inc., P. O. Box 6000, Sioux City, IA 51102-6000.

[5]Dickey-John GACII grain analysis computer, Dickey-John Corporation, P. O. Box 10, Auburn, IL 62615.

[6]SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513.

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Table 1.1. Grain sorghum seed sources and maturity for 85 genotypes used in the greenhouse study to evaluate the differential sorghum response to POST mesotrione[a].

Hybrid	ID_{50}	GR_{50}	Hybrid	ID_{50}	GR_{50}
	g/ha			g/ha	
DKS35-70	184	209	A 110	100	101
F222E	175	205	780B	99	84
Seneca	174	246	MG4748	99	102
KS310	156	121	DKS53-11	99	104
MG4665	153	105	GE-4532	98	104
3552	140	161	DKS54-00	98	105
A 121	136	194	DKS36-16	97	109
Pulsar	135	138	NK7829	96	92
F270E	134	195	5360	96	150
664	134	124	NK6673	96	106
764B	132	105	722B	95	109
F305C	131	109	697	95	131
GE-5615	127	149	F303C	94	96
775	126	155	8R18	94	104
NK7655	124	196	GWX2045	94	103
7B51	122	122	DK-44	94	105
672	121	168	T-38GS	93	106
A137	118	102	720B	92	99
7B47	117	143	GW 1467	92	151
NK4420	116	144	MG4772	91	108
DKS42-20	115	95	T-36GS	91	124

Table 1.1. Cont.

Hybrid	ID_{50}	GR_{50}	Hybrid	ID_{50}	GR_{50}
	g/ha			g/ha	
627	113	118	751B	91	122
DSS B6506	112	94	TR 442	91	103
7R34	111	107	NK3303	88	88
M3838	111	104	A 567	87	104
737	111	111	O-595	86	106
O-567	110	114	1G600	86	109
NK7633	110	100	766B	86	143
GWX3045	110	106	752B	85	108
GWX1445	109	103	O-530	85	102
7C22	108	116	TR 463	85	84
TR 434	108	113	6B50	85	105
85G46	108	105	NK5418	85	117
DKS37-07	108	105	O-525	84	92
84G50	106	110	KS 585	84	97
TR 481	106	151	5401	83	106
5B89	103	114	733Y	81	114
K73-J6	102	86	7R83	80	104
DSS B64	101	105	A 115C	79	92
85Y40	100	107	TR 438	78	104
5750	100	102	85G01	71	62
GW 1489	100	108	84G62	64	95
F505E	100	147			
LSD (0.05)	13	46		13	46

Table 1.2. Geographic location, soil type, percent organic matter, and soil pH for five sites in Kansas used to evaluate the differential sorghum response to POST mesotrione.

Site	Geographic location	Soil type	Soil taxonomic class	Percent organic matter	Soil pH
Belleville	North Central KS	Crete silt loam	Pachic Agriustolls	3.5	5.3
Garden City	Southwest KS	Keith silt loam	Aridic Agriustolls	1.6	8.5
Hays	West KS	Harney silt loam	Typic Agriustolls	2.0	6.0
Hesston	South Central KS	Ladysmith silty clay loam	Udertic Agriustolls	2.5	6.7
Manhattan	Northeast KS	Reading silt loam	Pachic Agriustolls	2.1	6.8

Table 1.3. POST mesotrione rate required to cause 50% visible injury (ID_{50}) and biomass reduction (GR_{50}) for 85 sorghum hybrids. Plants were treated at 3- to 5-leaf collar stage. Visible injury was assessed at 7 d after treatment and dry weights were determined 14 d after treatment.

Hybrid	ID_{50}	GR_{50}	Hybrid	ID_{50}	GR_{50}
	g/ha			g/ha	
DKS35-70	184	209	A 110	100	101
F222E	175	205	780B	99	84
Seneca	174	246	MG4748	99	102
KS310	156	121	DKS53-11	99	104
MG4665	153	105	GE-4532	98	104
3552	140	161	DKS54-00	98	105
A 121	136	194	DKS36-16	97	109
Pulsar	135	138	NK7829	96	92
F270E	134	195	5360	96	150
664	134	124	NK6673	96	106
764B	132	105	722B	95	109
F305C	131	109	697	95	131
GE-5615	127	149	F303C	94	96
775	126	155	8R18	94	104
NK7655	124	196	GWX2045	94	103
7B51	122	122	DK-44	94	105
672	121	168	T-38GS	93	106
A137	118	102	720B	92	99
7B47	117	143	GW 1467	92	151
NK4420	116	144	MG4772	91	108
DKS42-20	115	95	T-36GS	91	124

Table 1.3. Cont.

Hybrid	ID_{50}	GR_{50}	Hybrid	ID_{50}	GR_{50}
	g/ha			g/ha	
627	113	118	751B	91	122
DSS B6506	112	94	TR 442	91	103
7R34	111	107	NK3303	88	88
M3838	111	104	A 567	87	104
737	111	111	O-595	86	106
O-567	110	114	1G600	86	109
NK7633	110	100	766B	86	143
GWX3045	110	106	752B	85	108
GWX1445	109	103	O-530	85	102
7C22	108	116	TR 463	85	84
TR 434	108	113	6B50	85	105
85G46	108	105	NK5418	85	117
DKS37-07	108	105	O-525	84	92
84G50	106	110	KS 585	84	97
TR 481	106	151	5401	83	106
5B89	103	114	733Y	81	114
K73-J6	102	86	7R83	80	104
DSS B64	101	105	A 115C	79	92
85Y40	100	107	TR 438	78	104
5750	100	102	85G01	71	62
GW 1489	100	108	84G62	64	95
F505E	100	147			
LSD (0.05)	13	46		13	46

Table 1.4. Visible mesotrione injury of four sorghum hybrids with POST mesotrione application 1 and 2 weeks after treatment at five sites in Kansas.

		1 WAT[a]					2 WAT				
Hybrid	Rate	Belleville	Garden City	Hays	Hesston	Manhattan	Belleville	Garden City	Hays	Hesston	Manhattan
	g ai/ha			%					%		
7R83	0	0	0	0	0	0	0	0	0	0	0
	52	9	1	9	4	1	1	0	0	0	0
	105	16	14	15	15	6	4	4	4	3	3
	157	29	11	23	25	11	11	3	8	11	7
	210	33	11	31	32	15	15	1	8	10	9
84G62	0	0	0	0	0	0	0	0	0	0	0
	52	8	11	11	3	9	3	0	1	0	3
	105	11	10	20	24	11	6	5	7	13	6
	157	35	11	33	38	17	13	8	11	21	8
	210	28	19	38	37	24	13	9	14	17	10
85G01	0	0	0	0	0	0	0	0	0	0	0
	52	16	8	13	5	13	8	0	3	4	4
	105	18	11	25	23	25	10	1	11	19	10
	157	35	11	36	31	28	16	8	15	25	14
	210	34	23	41	38	33	19	16	17	29	17
Seneca	0	0	0	0	0	0	0	0	0	0	0
	52	4	8	4	0	3	2	1	0	0	0
	105	5	9	14	8	3	2	0	1	3	1
	157	10	9	16	14	8	5	4	2	5	3
	210	13	11	30	24	13	5	0	4	14	8
LSD(0.05)		7	7	7	7	7	6	6	6	6	6

[a]Abbreviations: WAT, weeks after treatment.

Table 1.5. Pearson correlation coefficients between visible mesotrione crop injury ratings 1 and 2 weeks after treatment and grain sorghum yield at four sites in Kansas in 2006.

	1 WAT[a]	2 WAT
Belleville	0.13 (P = 0.2)	0.15 (P = 0.2)
Hays	0.07 (P = 0.5)	0.10 (P = 0.3)
Hesston	0.12 (P = 0.3)	0.28 (P = 0.8)
Manhattan	0.30 (P = 0.3)	0.37 (P = 0.1)

[a]Abbreviations: WAT, weeks after treatment.

Chapter 2 - Effect of Postemergence Mesotrione Application Timing on Grain Sorghum

Abstract

Field experiments were conducted at Belleville, Colby, Hays, Hesston, Garden City, and Manhattan, KS, to determine grain sorghum response to POST application of mesotrione at three application timings. Mesotrione was applied at 52, 105, 157, and 210 g ai ha^{-1} in combination with 280 g ai ha^{-1} atrazine to grain sorghum at heights of 5 to 8, 15 to 20, and 30 cm, which correspond to early POST (EPOST), mid-POST (MPOST), and late POST (LPOST), respectively. All mesotrione rates caused visual injury at all application timings. Mesotrione applied at EPOST injured grain sorghum more than when applied at MPOST and LPOST timings. The EPOST application injured grain sorghum 19 to 88%, whereas injury from MPOST and LPOST application was 1 to 66% and 0 to 69%, respectively, depending on rate. Mesotrione injury was least at Belleville and most at the Hesston and Garden City (irrigated) sites regardless of growth stage. Correlation coefficient analysis indicated that observed mesotrione injury symptoms were not well correlated with sorghum yield; thus, mesotrione injury to grain sorghum did not influence grain yield. However, initial grain sorghum injury was severe, and this will likely be a major concern to producers.

Nomenclature: Mesotrione; sorghum, *Sorghum bicolor* (L.) Moench. SORBI.

Key words: HPPD-inhibiting herbicides, growth stages, herbicide timing, yield, injury.

Introduction

Weeds are one of the major obstacles in grain sorghum production (Hall and Bohner 2008). Competition from broadleaf weeds has been shown to reduce grain sorghum yields more than grass species or mixtures of broadleaf and grass weeds (Feltner et al. 1969). Some of the most common and troublesome broadleaf weeds in grain sorghum are *Amaranthus* spp. (Bridges 1992). Moore et al. (2004) reported that grain sorghum yields decreased 97 kg ha^{-1} for each increase of one Palmer amaranth (*Amaranthus palmeri* S. Wats.) plant per 15 m of row and decreased 392 kg ha^{-1} for each increase of 1 kg of Palmer amaranth dry matter per 15 m of row.

A major factor in the noxious nature of *Amaranthus* spp. is their ability to efficiently adapt to changes in cultural practices. For example, continuous use of herbicides with the same mode of action has resulted in development of resistance to photosystem II- (Anderson et al. 1996), acetolactate synthase (ALS)- (Horak and Peterson 1995), protoporphyrinogen oxidase (protox)- (Shoup et al. 2003), and enolpyruvyl-shikimate-phosphate synthase (EPSPS)- (Vencill et al. 2006) inhibiting herbicides in numerous populations of *Amaranthus* species such as Palmer amaranth, common waterhemp (*Amaranthus rudis* Sauer), redroot pigweed (*Amaranthus retroflexus* L.), and Powell amaranth (*Amaranthus powellii* S. Watson) (Anonymous 2008a; Culpepper et al. 2006; Heap 1997).

Mesotrione is a selective PRE and POST herbicide that effectively controls several broadleaf weeds, including *Amaranthus* spp. It also controls troublesome weeds such as photosystem II-, ALS-, protox-, and EPSPS-resistant *Amaranthus* spp. (Armel et al. 2003a; Stephenson et al. 2004; Sutton et al. 2002). Sorghum growers currently rely on

PRE applications of mesotrione, but without adequate precipitation to activate mesotrione, weed control may not be adequate (Armel et al. 2003b). POST application of mesotrione demonstrated consistent control of weeds but caused chlorosis in grain sorghum when applied at 70.5 g ha^{-1} (Horky and Martin 2005). Furthermore, Abit et al. (2009) observed differential response of grain sorghum hybrids to POST treatments of mesotrione; however, this information is of limited use because application was made only at the early seedling stage. Extensive research has not been conducted to evaluate the effect of mesotrione on grain sorghum growth and development at different stages. Proper timing of POST mesotrione application is essential to maximize weed control and reduce crop injury (Johnson et al. 2002). The objective of this research was to determine whether grain sorghum injury and grain yield are affected by mesotrione application timing.

Materials and Methods

Field experiments were conducted in 2007 at Kansas State University experiment fields in Belleville, Colby, Hays, Hesston, Garden City, and Manhattan. Two experiments were conducted at the Garden City site, one under irrigated and the other under dryland conditions. Experiments at all other sites were conducted under dryland conditions. Geographical location, soil type, soil taxonomic class, soil pH, and percentage organic matter are shown in Table 2.1. A mesotrione-susceptible grain sorghum hybrid, ‘Pioneer 84G62’, (Abit et al. 2009) was planted approximately 3 cm deep at 146,000 seeds ha^{-1}. The selection of a mesotrione-susceptible grain sorghum hybrid was to illustrate the worst-case scenario of grain sorghum plant response to mesotrione. Experiment plots were maintained weed-free with a PRE application of metolachlor plus atrazine at 1,412 + 1,121 g ai ha^{-1} and hand weeding as needed.

At each of the seven sites, the experiment was a randomized complete block design with a split plot arrangement. The whole-plot factor was application timing, and the split-plot factor was mesotrione rate. There were four replications at each site. Subplots consisted of four rows spaced at 0.76-m-wide that were 11.5 m long. Plots were randomly assigned to receive mesotrione treatments when sorghum was 5 to 8, 15 to 20, or 30 cm in height, which correspond to EPOST, MPOST, and LPOST. Mesotrione[1] was applied to subplots at 52, 105, 157, or 210 g ai ha^{-1} in combination with 280 g ai ha^{-1} atrazine at each application timing with a tractor-mounted sprayer calibrated to deliver 187 L ha^{-1} at 138 kPa. Atrazine was added to mesotrione treatments because mesotrione is commonly applied with atrazine under field conditions to increase control of some weed species (Johnson et al. 2002), therefore, the inclusion of attrazine in this study facilitates direct applicability to sorghum production. All spray mixtures included 1% (v/v) crop oil concentrate[2]. A nontreated control was included for comparison.

Sorghum plant injury was visually rated 1, 2, 4, and 8 WAT. Injury ratings were based on a scale of 0 (no injury) to 100 (plant death). Days to half bloom (DHB) and plant heights at flowering were recorded. The DHB data were gathered only at Hays, Hesston, and Manhattan. Sorghum grain was mechanically harvested from the two middle rows of each plot and weighed, and grain yields were adjusted to 14% moisture content.

Data were analyzed in a mixed linear model by using the MIXED procedure of SAS 9.1[3]. Site, application timing, herbicide rate, and interactions between these factors were considered fixed effects, and block (nested within site) and interactions with block were considered random effects. Mean comparisons were made by using Fisher's Protected LSD test at P = 0.05. In addition, orthogonal contrast (P = 0.05) was used to compare yields between mesotrione-treated and nontreated means. Regression analyses were performed using Sigma Plot 10[4] procedures to evaluate the relationship between grain sorghum injury and herbicide rate. Homogeneity of variance was tested, and crop injury data were subjected to an arcsine transformation because of unequal variances (Kuehl 2000). Interpretations were not different from the nontransformed data; therefore, the nontransformed data are presented. Correlation coefficient analysis on injury versus yield was done by using PROC CORR of SAS 9.1.

Results and Discussion

Grain Sorghum Injury

There was a significant timing by rate by site interaction (P = < 0.0001); therefore, data were analyzed and are presented by site. Crop injury data were collected 1, 2, 4, and 8 WAT; however, data presented are only for 1 and 4 WAT because greatest injury and grain sorghum recovery respectively was observed for most treatments on those dates.

Mesotrione injured sorghum at all rates and application timings at all sites. Injury symptoms were characterized by leaf chlorosis and bleaching followed by necrosis of the tissue. Chlorosis and bleaching started at the apical and intercalary meristematic zones of the internodes and leaves, including leaf veins, and become progressively necrotic 1 WAT (Figure 2.1). Concurrently, stunting was observed and intensified with time. By 4

WAT, plants partially recovered from injury (Figure 2.2). Recovery from injury decreased as mesotrione application rate increased. At 1 WAT, visible injury was greatest at the Hesston and Garden City (irrigated) sites and least at the Belleville site (Figure 2.1). Grain sorghum injury differences among sites may be due to the crop growth conditions and stand during application. For example, plants at the Belleville site were more uniform and vigorous, which may be in part due to a higher organic matter content compared to other sites (Table 2.1).

Significant timing by rate interaction effects were observed at all sites except Hesston and Garden City (irrigated) at 1 WAT and Hays and Manhattan at 4 WAT (data not shown). Overall, injury symptoms were more severe when mesotrione was applied at EPOST than at MPOST or LPOST. The EPOST application injured grain sorghum 19 to 88%, whereas injury from MPOST and LPOST was 1 to 66% and 0 to 69%, respectively, depending on rate. This suggests that younger grain sorghum is more likely to be injured by mesotrione than more developed sorghum. Mesotrione application at the 105 g ha^{-1} rate caused 28 to 69% injury at EPOST, but injury decreased 3 to 52% and 4 to 33% when sorghum was treated at MPOST and LPOST, respectively. These results are in agreement with previous research that showed greater sorghum injury from EPOST applications of mesotrione (Miller and Regehr 2002). At 4 WAT, plants treated with 52 and 105 g ha^{-1} mesotrione at MPOST and LPOST generally recovered and produced new normal shoots, whereas plants treated at EPOST continued to show injury symptoms regardless of rate (Figure 2.2). However, all plants appeared normal by the end of the growing season (data not shown). Observed crop injury at 4 WAT from EPOST application may be due to greater initial injury of the plants, which led to longer recovery.

Sorghum injury from mesotrione increased with increased rate. In general, injury symptoms were greatest in plants treated with 210 g ha^{-1} mesotrione and least in those treated with the 52 g ha^{-1} rate. Averaged across stages and sites, sorghum injury was significantly greatest when mesotrione was applied at 210 g ha^{-1} (53%) and least when it was applied at 52 g ha^{-1} (22%) (data not shown).

Agronomic Response

Plant height was similar when mesotrione was applied at all rates and growth stages (data not shown). In addition, no treatment by site interaction was observed. A significant timing by rate by site interaction for DHB was observed; therefore, data were analyzed and presented by site. DHB of sorghum plants treated with mesotrione was similar when mesotrione was applied at MPOST and LPOST; however, significant delays were observed when mesotrione was applied at EPOST (Table 2.2). DHB was affected when mesotrione was applied at rates greater than 52 g ha^{-1} at EPOST. Delays of 3 to 6, 5 to 9, and 7 to 9 d were observed when mesotrione was applied at 105, 157, and 210 g ai ha^{-1}, respectively. The combination of mesotrione-susceptible grain sorghum hybrid, mesotrione application at an early growth stage, and greater mesotrione rates increased the risk of late bloom, which may require a longer growing season to allow grain filling. In areas where time of planting is not critical, delayed maturity would not be much of a concern. In Kansas, however, time of planting date is dictated by weather and any maturity delay would likely impair harvest.

There was no significant timing by rate by site and timing by rate interactions for grain yield; therefore, yield data were averaged over rates. Sorghum grain yields ranged from 4,237 to 8,884 kg ha^{-1} and 4,027 to 8,572 kg ha^{-1} for the nontreated and mesotrione-treated plots, respectively (Table 2.3). In general, grain yields were lower in mesotrione-treated plots than in the nontreated control. Differences in grain sorghum yields were observed among mesotrione application timings (Table 2.4).   EPOST timing showed more yield reduction than MPOST in three out of seven locations, and more than LPOST in two out of seven locations. Yield reduction in MPOST and LPOST timings were equal in all sites. Among all sites, Hesston showed an unusually high grain yield reduction, as much as 43% at EPOST, which was likely enhanced by moisture deficit resulting from below-normal precipitation and above-normal temperatures during, boot, bloom, and soft dough stages of the crop (Anonymous 2008b). These extreme environmental conditions during critical reproductive stages of grain sorghum development can reduce flower numbers, pollination, and translocation of assimilates to grain, collectively reducing grain yield (Boyer 1982; Taiz and Zeiger 2006).

The effect of mesotrione application on grain sorghum yield is a major consideration for producers. Correlation coefficient analyses indicated that injury caused by mesotrione was poorly correlated with grain sorghum yield (Table 2.5), suggesting that the observed level of mesotrione-induced injury to grain sorghum, regardless of mesotrione application timing, is transient and therefore sorghum plants can sustain some level of injury without reductions in grain yield. However, risks and benefits of practices that can adversely affect the crop physical condition should also be considered. Crop aesthetics are important not only to producers, but also to land owners who rent land for crop production. Although weed size should be the primary criteria for POST herbicide application timing, when producers have some flexibility concerning weed size in mesotrione application timing, MPOST or LPOST mesotrione applications may be preferred over EPOST applications because of reduced visible injury.

Sources of Materials

[1]Mesotrione, Callisto® herbicide, Syngenta Crop Prtection, Inc., Greensboro, NC 27419-8300.

[2]Prime Oil, Terra International Inc., P.O. Box 6000, Sioux City, IA 51102-6000.

[3]SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513.

[4]Systat Software, Inc. 501 Canal Blvd., Suite E, Point Richmond, CA 94804-2028.

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Table 2.1. Geographic location, soil type, taxonomic class, percentage organic matter, and soil pH for the experimental sites used in this study.

Site	Geographic location	Planting date	Soil type	Soil taxonomic class	% Organic matter	Soil pH
Belleville	North central KS	June 5, 2007	Crete silt loam	Pachic Agriustolls	4.4	6.8
Colby	Northwest KS	May 29, 2007	Keith silt loam	Aridic Agriustolls	2.8	6.1
Garden City (irrigated)	Southwest KS	June 7, 2007	Manter coarse loam	Aridic Agriustolls	0.8	7.7
Garden City (dryland)	Southwest KS	June 7, 2007	Richfield silt loam	Aridic Agriustolls	1.5	8.0
Hays	West KS	June 7, 2007	Crete silt loam	Pachic Agriustolls	1.7	6.6
Hesston	South central KS	June 21, 2007	Ladysmith silty clay loam	Udertic Agriustolls	2.2	6.6
Manhattan	Northeast KS	June 14, 2007	Reading silt loam	Pachic Agriustolls	2.0	6.5

Table 2.2. Days to half bloom of grain sorghum plants as influenced by POST mesotrione application timing at Hays, Hesston, and Manhattan, KS.

		Days to half bloom		
Timing[a]	Rate	Hays	Hesston	Manhattan
	g ai/ha			
EPOST	Nontreated check	67	68	66
	52	69	70	67
	105	73	71	69
	157	76	73	71
	210	76	74	73
MPOST	Nontreated check	69	66	67
	52	67	65	67
	105	70	65	67
	157	71	65	68
	210	69	65	68
LPOST	Nontreated check	69	65	68
	52	67	64	66
	105	66	64	67
	157	67	64	67
	210	67	64	68
LSD (0.05)		3	2	2

[a] EPOST, early POST when sorghum plants were 5 to 8 cm tall; MPOST, mid-POST when sorghum plants were 15 to 20 cm tall, LPOST, late POST when sorghum plants were at 30 cm tall.

Table 2.3.Yield comparison of nontreated and mesotrione treated grain sorghum plants as influenced by POST mesotrione application timing at Belleville, Colby, Hays, Hesston, Garden City and Manhattan, KS.

		Yield						
Timing[a]	Treatment	Belleville	Colby	Hays	Hesston	Garden City (irrigated)	Garden City (dryland)	Manhattan
					kg/ha			
EPOST	Nontreated	4237	7416	8591	4815	5756	4775	8069
	Treated	4069	6746	7901	2725	5841	4756	7181
p-value		0.0002	NS	0.0059	0.0007	NS	NS	0.0176
MPOST	Nontreated	4296	7050	8019	5193	5129	5172	8518
	Treated	4027	7284	7521	4795	5130	5636	8213
p-value		<0.0001	NS	0.0052	NS	NS	NS	NS
LPOST	Nontreated	4282	7467	8884	5528	5114	5852	8423
	Treated	4039	7293	8572	4797	5043	5802	7900
p-value		<0.0001	NS	0.0463	NS	NS	NS	NS

[a] EPOST, early POST when sorghum plants were 5 to 8 cm tall; MPOST, mid-POST when sorghum plants were 15 to 20 cm tall, LPOST, late POST when sorghum plants were at 30 cm tall.

Table 2.4. Yield reduction of grain sorghum plants as influenced by POST mesotrione application timing at Belleville, Colby, Hays, Hesston, Garden City, and Manhattan, KS.

Timing[a]	Yield reduction						
	Belleville	Colby	Hays	Hesston	Garden City (irrigated)	Garden City (dryland)	Manhattan
	%						
EPOST	4	9	8	43	0	0	11
MPOST	6	0	6	8	0	0	4
LPOST	6	2	4	13	1	1	6
LSD (0.05)	NS	7	NS	25	NS	NS	6

[a] EPOST, early POST when sorghum plants were 5 to 8 cm tall; MPOST, mid-POST when sorghum plants were 15 to 20 cm tall, LPOST, late POST when sorghum plants were at 30 cm tall.

Table 2.5. Pearson correlation coefficients between visible mesotrione injury ratings 1 wk after treatment and grain sorghum yield at Belleville, Colby, Hays, Hesston, garden City, and Manhattan, KS.

	Correlation coefficients						
Timing[a]	Belleville	Colby	Hays	Hesston	Garden City (irrigated)	Garden City (dryland)	Manhattan
EPOST	-0.47 (P = 0.73)	-0.47 (P = 0.06)	-0.35 (P = 0.13)	-0.56 (P = 0.10)	0.04 (P = 0.86)	-0.03 (P = 0.89)	-0.52 (P = 0.33)
MPOST	-0.28 (P = 0.22)	-0.15 (P = 0.52)	-0.23 (P = 0.32)	-0.17 (P = 0.47)	-0.45 (P = 0.06)	0.26 (P = 0.26)	-0.35 (P = 0.12)
LPOST	-0.34 (P = 0.14)	0.14 (P = 0.55)	-0.28 (P = 0.23)	-0.35 (P = 0.13)	-0.37 (P = 0.11)	0.05 (P = 0.84)	-0.58 (P = 0.10)

[a] EPOST, early POST when sorghum plants were 5 to 8 cm tall; MPOST, mid POST when sorghum plants were 15 to 20 cm tall, LPOST, late POST when sorghum plants were at 30 cm tall.

Figure 2.1. Visible injury on grain sorghum plants 1 wk after treatment as influenced by POST mesotrione application timing at Belleville, Colby, Hays, Hesston, Garden City and Manhattan, KS. EPOST, early POST when sorghum were 5 to 8 cm tall; MPOST, mid-POST when sorghum were 15 to 20 cm tall; LPOST, late POST when sorghum plants were at 30 cm tall.

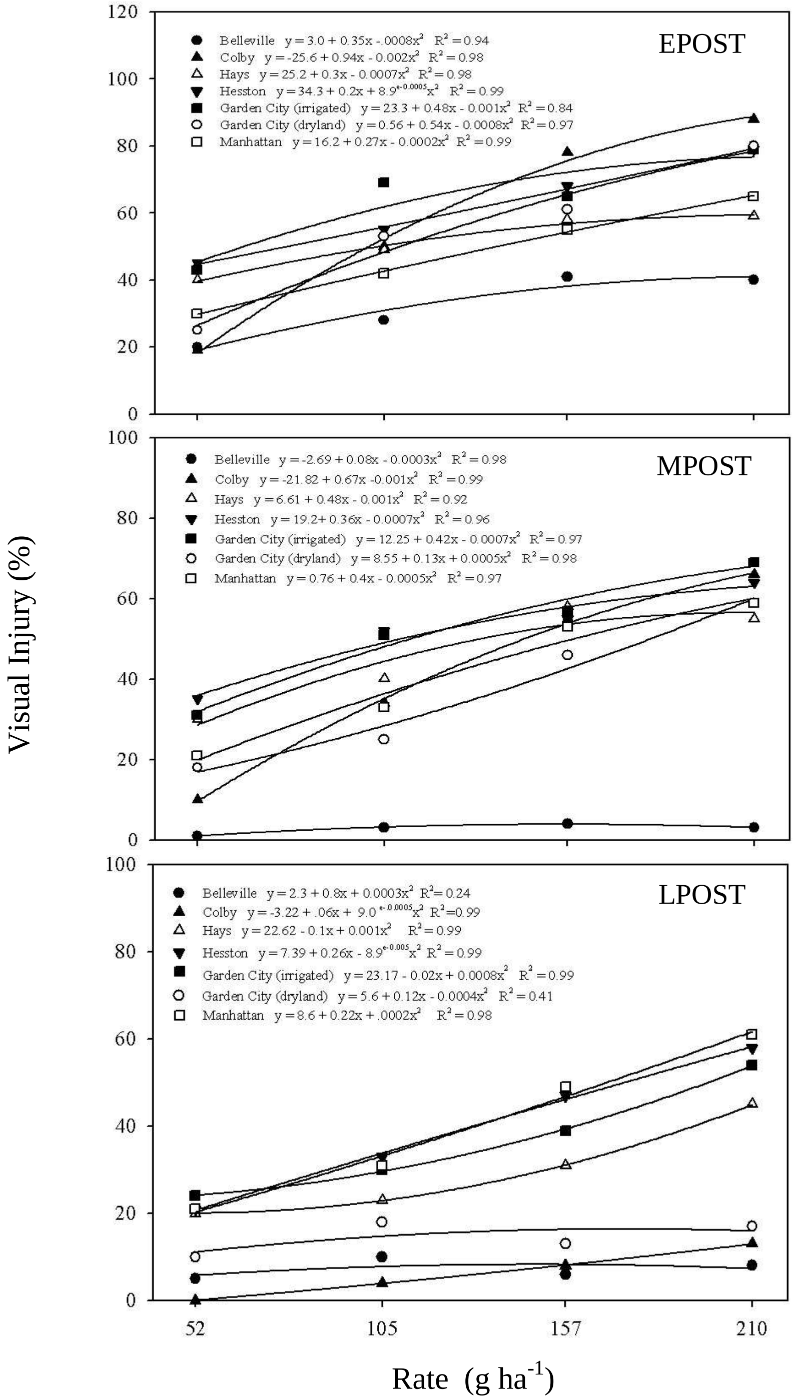
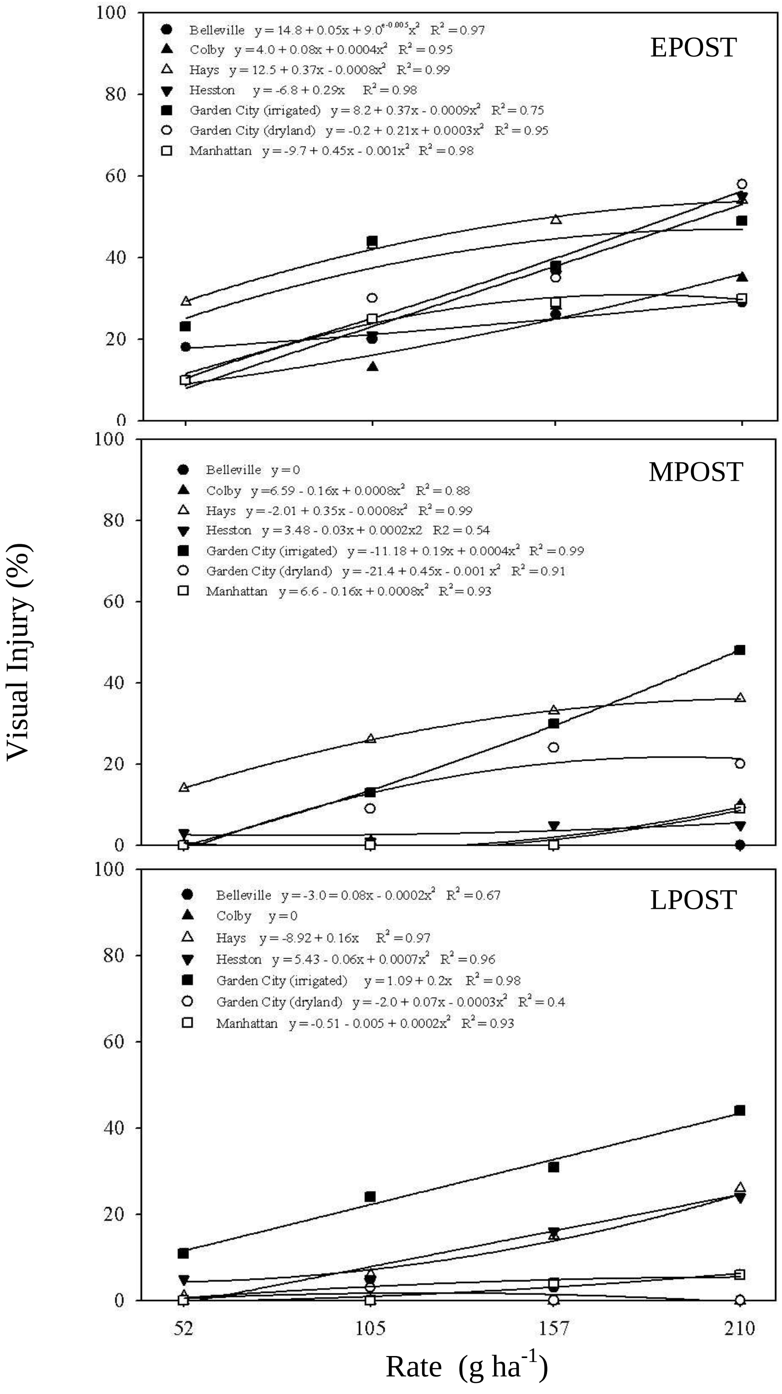


Figure 2.2. Visible injury on grain sorghum plants 4 wk after treatment as influenced by POST mesotrione application timing at Belleville, Colby, Hays, Hesston, Garden City and Manhattan, KS. EPOST, early POST when sorghum were 5 to 8 cm tall; MPOST, mid-POST when sorghum were 15 to 20 cm tall; LPOST, late POST when sorghum plants were at 30 cm tall.



Chapter 3 - Absorption, Translocation, and Metabolism of Mesotrione in Grain Sorghum

Abstract

Studies were conducted under controlled growth chamber conditions to determine if differential absorption, translocation, or metabolism were the basis for the differential response of grain sorghum hybrids to mesotrione. Mesotrione-tolerant (‘Dekalb DKS35-70’) and -susceptible (‘Pioneer 84G62’) sorghum grain hybrids were treated with ^{14}C-labeled mesotrione. At 1 d after treatment (DAT), absorption was 7% in both hybrids; at 7 DAT, however, absorption remained near steady in Pioneer 84G62 but increased to 12% in Dekalb DKS35-70. Translocation of ^{14}C-mesotrione in sorghum hybrids was similar with less than 30% of the absorbed herbicide translocated out of the treated leaf by 7 DAT. A distinct metabolite of ^{14}C-mesotrione was separated in both hybrids at 3 DAT. The amount of mesotrione parent compound that remained in Pioneer 84G62 and DKS35-70 was 72 and 65%, respectively. Dekalb DKS35-70 had significantly less mesotrione at 3 DAT than Pioneer 84G62 did, but the amount of mesotrione was similar for both hybrids at 5 and 7 DAT. Rapid metabolism of mesotrione may help explain the differential response of grain sorghum hybrids.

Nomenclature: Mesotrione; sorghum, *Sorghum bicolor* (L.) Moench. SORBI.

Key words: HPPD-inhibiting herbicides, hybrids.

Introduction

Mesotrione is a selective, systemic, soil- and foliar-applied herbicide that controls broadleaf and some grass weeds, such as Palmer amaranth (*Amaranthus palmeri* S. Wats.) and common waterhemp (*Amaranthus rudis* Sauer), in corn (*Zea mays* L.), including weeds that are resistant to photosystem II-, acetolactate synthase, protoporphyrinogen oxidase, and 5-enopyruvyl-shikimate-3-phosphate synthase herbicides (Anderson et al. 1996; Horak and Peterson 1995; Shoup et al 2003; Vencill et al. 2006). Mesotrione is a competitive inhibitor of the enzyme *p*-hydroxyphenylpyruvate dioxygenase (HPPD), which catalyzes the conversion of tyrosine to plastoquinone and α-tocopherol (Mitchell et al. 2001, Norris et al. 1998) resulting in carotenoid biosynthesis reduction. Mesotrione is absorbed rapidly by susceptible species following foliar application, and is translocated acropetally and basipetally (Mitchell at al. 2001).

Mesotrione injury symptoms in susceptible plants include bleaching followed by necrosis within 3 to 5 d (Senseman 2007). Bleaching symptoms result from inhibition of carotenoid biosynthesis, coupled with destruction of chlorophyll by light (photooxidation) and inhibition of chlorophyll biosynthesis (Hess 2000; Kim et al. 2001). Under high light intensities, rapidly growing species use ≤ 50% of absorbed light energy, and the remaining absorbed light is excess energy (Demmig-Adams et al. 1996). Plants have natural ability to dissipate this excess energy through photoprotection by carotenoids (Taiz and Zeiger 2008). When chlorophyll is electronically excited by absorbing light photons, it is transformed from a ground state short-lived, singlet form to an excited state, longer-lived, triplet form (Hess 2000). If the excited state of chlorophyll is not rapidly quenched, it can react with molecular oxygen to form singlet oxygen. The extremely reactive, singlet oxygen then reacts with, and damages, many cellular components (Muller et al. 2001; Taiz and Zeiger 2008). Carotenoids exert their photoprotective action by rapidly quenching the excess energy of the triplet chlorophyll, which is especially generated under high light intensity. If carotenoid synthesis is inhibited, chlorophyll and photosynthetic membrane destruction occurs because of the plant's inability to quench the reactive, oxidative energy (Hess 2000).

Currently, sorghum growers rely on PRE applications of mesotrione to control *Amaranthus* species that are resistant to several herbicide chemistries and to control many other weeds commonly found in grain sorghum; however, without sufficient moisture to activate mesotrione, weed control may not be adequate (Armel et al. 2003). POST application of mesotrione consistently controlled weeds but caused bleaching and chlorosis in grain sorghum (Abit et al. 2009; Horky and Martin 2005). Research has demonstrated, however, grain sorghum hybrids differ in tolerance to POST applications of mesotrione. Abit et al. (2009) reported that among 85 sorghum hybrids evaluated, 23 were susceptible, 45 were intermediate, and 17 were tolerant to mesotrione. Furthermore, the mesotrione rate that caused 50% sorghum injury ranged from 121 to 184 g ha^{-1} and from 64 to 91 g ha^{-1} for tolerant and susceptible hybrids, respectively. In general, tolerant hybrids showed less injury and recovered more rapidly from mesotrione injury than susceptible hybrids. In corn, mesotrione tolerance has been attributed to lower absorption and increased cytochrome P_{450}-mediated metabolism compared with susceptible weed species (Bartlett and Hall 2000; Mitchell et al. 2001). However, no research has been conducted to examine foliar absorption, translocation and metabolism of mesotrione in grain sorghum. Therefore, the objective of this study was to determine if absorption, translocation and metabolism was the basis for the differential response of grain sorghum hybrids to mesotrione.

Materials and Methods

Plant Materials

Mesotrione-tolerant ('Dekalb DKS35-70') and mesotrione-susceptible ('Pioneer 84G62') grain sorghum hybrids (Abit et al. 2009) were planted in separate 11-cm diameter containers filled with sand:Morrill loam (fine-loamy, mixed, mesic Typic Arguidolls) soil (1:1 by vol) with pH 6.5 and 2% organic matter. Plants were grown under growth chamber conditions with 30/25 C day/night temperatures and a 16-h photoperiod with supplemental light intensity of 250 μmol m^{-2} s^{-1} photosynthetic photon flex density. Plants were watered as needed and fertilized weekly with a commercial fertilizer[1] solution containing 1.2 g L^{-1} total nitrogen, 0.4 g L^{-1} phosphorus, and 0.8 g L^{-1} potassium. After emergence, grain sorghum hybrid seedlings were thinned to 2 plants pot^{-1}.

Absorption and Translocation

At the four-leaf stage, plants were treated with 10, 1-μl droplets of ^{14}C-labeled mesotrione [phenyl-U-^{14}C]-mesotrione with specific activity of 781 MBq g^{-1}, on the upper surface of the third leaf of both mesotrione-susceptible and mesotrione-tolerant plants. A single 1-μl droplet contained 87 Bq of ^{14}C-mesotrione. Unlabeled mesotrione was added to the radioactive solution to obtain 105 g ai ha^{-1}, in a carrier volume of 187 L ha^{-1}. Crop oil concentrate (COC)[2] was added at 1% v/v to enhance droplet-to-leaf surface contact. Plants were harvested at 1, 3 and 7 d after treatment (DAT) and were divided into six sections: treated leaf, leaves above the treated leaf, stem above the treated leaf, leaves below the treated leaf, stem below the treated leaf, and roots. Treated leaves were washed with 15 ml of a 75% methanol solution for 20 s to remove any unabsorbed herbicide. Radioactivity in the leaf rinsate was measured by using liquid scintillation spectrometry (LSS)[3]. Plant sections were dried at 45 C for 48 h and then combusted using a biological oxidizer[4]. Radioactivity recovered for each plant part was measured by using LSS. Herbicide absorption was calculated by dividing the radioactivity recovered in the entire plant by the total radioactivity applied to the plant. Herbicide translocation was calculated by dividing the radioactivity recovered in each plant part by the total radioactivity absorbed in the plant (Schuster et al. 2007).

Mesotrione Metabolism

To detect all metabolites, higher mesotrione radioactivity was used in this study compared with the absorption and translocation study. Ten 1-μl droplets containing 2,183 Bq of ^{14}C-mesotrione were applied to the upper surface of the four largest leaves on each plant in a container. Unlabeled mesotrione was mixed with ^{14}C-mesotrione to reach the desired application rate as described in the foliar absorption and translocation study. Herbicide solution included COC, as previously described.

Treated leaves were harvested at 3, 5, and 7 DAT. The leaves were washed with 15 ml 75% methanol to remove any unabsorbed herbicides. Plants tissues were then frozen with liquid nitrogen and ground with a mortar and pestle. Subsamples of the ground tissue were weighed and oxidized, and captured $^{14}CO_2$ was measured using LSS to assess the amount of radioactivity in the plant tissue. Leaf tissues were stored at -80 C until radioactivity was extracted.

Frozen leaf tissues were homogenized with 20 ml of 75% methanol (by vol) and shaken for 1 h. Samples were filtrated and the supernatant was saved. The leaf tissues were resuspended twice in 15 ml of 50% methanol and shaken for an additional hour. Samples were filtered, and supernatant was added to the first and second supernatant. The remaining leaf tissues were resuspended in 15 ml of 100% methanol and shaken for 6 h. Samples were filtered, and the supernatant was added to the total supernatant. To determine the amount of radioactivity not extracted into the supernatant, the remaining plant residue and filter paper were oxidized, and radioactivity was measured (^{14}C extraction efficiency = 95.3 ± 0.2). Supernatant was then evaporated at 35 C to 0.5 ml using a centrivap[5]. Solution was then filtered with a 0.2 μm filter[6] and stored at -20 C until use.

Extracts were injected into a Beckman high-performance liquid chromatograph[7] equipped with a Zorbax ODS endcapped Sb-C18 column[8] (4.6 x 250 mm, 5μm particle size) with a mobile phase of water with 0.1% formic acid and methanol at a flow rate of 0.5 ml min^{-1} and an injection volume of 50 μl. The elution profile was as follows: step 1, 40% methanol isocratic gradient for 6 min; step 2, 40 to 75% methanol linear gradient for 2 min; step 3, 75 to 100% methanol linear gradient for 2 min; step 4, 100% methanol isocratic gradient for 3 min; step 5, 100 to 40% methanol linear gradient for 3 min; and step 6, 40% methanol isocratic gradient for 7 min. Fractions were sequentially collected at 0.5-min intervals, and radioactivity was measured by using LSS. A mesotrione standard was included to determine the herbicide retention time.

Experimental Design and Data Analysis

The experiment design for all studies was a randomized complete block. Treatments were blocked by harvest time. Foliar absorption and translocation treatments were replicated four times, and the experiment was conducted three times. In the metabolism study, the treatments were replicated four times, and the experiment was repeated. There were no interactions among runs for either study; therefore, data were pooled over runs. Data from both studies were analyzed using ANOVA, and means was separated using standard errors at $P \leq 0.05$ (Schuster et al. 2007).

Results and Discussion

Absorption

Absorption of ^{14}C mesotrione was low in both grain sorghum hybrids (Table 3.1) and lower than mesotrione absorption in corn reported by others (Armel, et al. 2004). The low foliar mesotrione absorption in sorghum may be due to the presence of a large number of prickle hairs (trichomes with swollen bases and sharp tips) and higher amount of loosely bound leaf wax (Cannon and Kummerow 1957; Traore et al. 1989). For example, wax concentration in sorghum leaves was 0.6% but was only 0.35% in corn (Cannon and Kummerow 1957). At 1 DAT, both mesotrione-tolerant (DKS35-70) and mesotrione-susceptible (84G62) hybrids absorbed 7% of the total applied mesotrione. Mesotrione absorption in DKS35-70 increased over time but peaked 3 DAT in 84G62. At 3 and 7 DAT, DKS35-70 absorbed 9 and 12%, respectively, whereas 84G62 absorbed only 8% at both harvest times. Other researchers have reported similar amounts of foliar absorption of other POST HPPD herbicides in other species (Young and Hart 1998). The tolerant sorghum hybrid had slightly higher absorption than the susceptible hybrid, likely because there was less mesotrione injury to the tolerant hybrid. As a consequence, tolerant tissue would continue absorbing herbicide over time, whereas the susceptible tissue would be severely injured preventing further mesotrione absorption (Devine et al. 1993).

Translocation

Mesotrione translocation out of the treated leaf was similar in tolerant and susceptible sorghum hybrids at each harvest time (P = 0.99); therefore, data were averaged across hybrids. Translocation of ^{14}C mesotrione in sorghum was relatively low (Table 3.2). A similar level of translocation was reported when mesotrione was applied to corn and soybean [*Glycine max* (L.) Merr] foliage (Armel et al. 2004; Mitchell 2001; Schuster et al. 2007). Only 10 to 17% of ^{14}C mesotrione translocated to the rest of the foliage with 7 to 11% to the stem and 5% or less to the roots (Table 3.2). No more than 30% of the absorbed ^{14}C mesotrione translocated out of the treated leaf by 7 DAT. At 7 DAT, most of the ^{14}C mesotrione remained in the treated leaf. These results are in agreement with earlier research that showed that the bulk of the ^{14}C mesotrione applied to Canada thistle [*Cirsium arvense* (L.) Scop] remained in the treated leaf; only 9 to 20% of ^{14}C mesotrione translocated to the rest of the foliage, and 2% of less translocated to the roots (Armel et al. 2005). Mesotrione translocation to the different plant parts, however, was different between harvest timings. At 1 DAT translocation of ^{14}C mesotrione to the leaves above the treated leaf was 8%, whereas at 3 and 7 DAT translocation was 16%.

Mesotrione Metabolism

A distinct metabolite was isolated in both hybrids 3 DAT. At 5 DAT, two metabolites were separated in both hybrids, whereas three and two metabolites were segregated from the parent herbicide at 7 DAT in DKS35-70 and 84G62, respectively (Table 3.3). Previous metabolism studies in plants and soil show degradates can be formed from mesotrione with MNBA [4-(methylsufonyl)-2-nitrobenzoic acid] and AMBA [2-amino-4-(methylsulfonyl) benzoic acid] as the major metabolites (Alferness and Wiebe 2002; Armel et al. 2005). The mesotrione metabolites were eluted at 7, 9.5 and 14 min during the elution profile. Based on the mobile phase gradient used, of the three metabolites, the first two appear to be hydrophobic, and the third appears to be hydrophilic. DKS35-70 had significantly less mesotrione at 3 DAT than 84G62 had. At 3 DAT, 72% of mesotrione remained in 84G62; only 65% remained in DKS35-70. These results are similar to those of Wichert et al. (1999), who found that sweet corn cultivars that are more tolerant to POST applications can metabolize mesotrione more rapidly than susceptible cultivars. Although there was a considerable amount of mesotrione present at 3 DAT, previous study revealed that differences in injury were still observed between the two hybrids (Abit et al. 2009). Considering the rate of absorption (9%) and translocation (30% of the absorbed mesotrione), the difference in the amount of mesotrione retained (percentage of the translocated amount) in tolerant and susceptible hybrids can cause significant differences in injury. The greater mesotrione metabolism in tolerant, rather than in susceptible, sorghum hybrids resulted in lower concentration of mesotrione in plants, which led to earlier recovery in the tolerant sorghum. The metabolism pattern of mesotrione, however, was similar for both hybrids at 5 and 7 DAT. At 5 DAT, 59 and

63% of the mesotrione remained in DKS35-70 and 84G62 hybrids, respectively, whereas 36 and 43% of the mesotrione remained in DKS35-70 and 84G62, respectively, 7 DAT.

Because no differences in foliar absorption and translocation were observed between hybrids, selectivity is probably not due to differential absorption or translocation. Previous researchers have identified herbicide metabolism as the primary basis for differential response of crops to mesotrione (Barlett and Hall 2000; Mitchell et al. 2001). The tolerance to mesotrione treatment in the tolerant hybrid could result from the slightly more rapid metabolism in this hybrid. Tolerant species have the capacity to metabolize herbicide more rapidly and extensively than susceptible species. Thus, rapid metabolism may help explain the differential response of grain sorghum hybrids to mesotrione observed in this study.

Sources of Materials

[1]Miracle-Gro soluble fertilizer, Scotts Miracle-Gro Products Inc., 1411 Scottslawn Road, Marysville, OH 43041.

[2]Prime Oil, Terra International Inc., P.O. Box 6000, Soiux City, IA 51102-6000.

[3]Tricarb 2100TR Liquid Scintillation Analyzer, Packard Instrument Co., 800 Research Parkway, Meriden, CT 06450.

[4]R. J. Harvey Biological Oxidizer, Model OX-600, R. J. Harvey Instrument Co., 123 Patterson Street, Hillsdale, JN 07642.

[5]Centrivap, Labconco, 8811 Prospect, Kansas City, MO 64132.

[6]0.2-µm filter, Osmotics Inc., 5951 Clearwater Drive, Minnetonka, MN 55343.

[7]Beckman high performance liquid chromatograph, Beckman Coulter Inc., Life Science Division, 4300 N. Harbor Boulevard, P.O. Box 3100, Fullerton, CA 92834-3100.

[8]Zorbax ODS endcapped Sb-C18 column, Agilent Technologies, Chemical Analysis Group, 2950 Centerville Road, Wilmington, DE 19808.

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Table 3.1. Absorption of mesotrione in mesotrione-tolerant (DKS35-70) and mesotrione-susceptible (84G62) grain sorghum hybrids at 1, 3, and 7 d after treatment (DAT)[a].

Hybrid	1 DAT	3 DAT	7 DAT
		% absorbed	
DKS35-70	7 ± 1	9 ± 1	12 ± 2
84G62	7 ± 1	8 ± 1	8 ± 1

[a]Table values are means ± standard error.

Table 3.2. Translocation of mesotrione in grain sorghum hybrids at 1, 3, and 7 d after treatment (DAT). Means are the average of two hybrids[a].

Plant part	1 DAT	3 DAT	7 DAT
		% translocated	
Treated leaf	76 ± 2	71 ± 2	71 ± 2
Leaves above treated leaf	8 ± 1	16 ± 1	16 ± 1
Stem above treated leaf	2 ± 0	2 ± 0	2 ± 0
Leaves below treated leaf	2 ± 1	1 ± 0	1 ± 0
Stem below treated leaf	9 ± 1	6 ± 0	5 ± 1
Roots	3 ± 2	4 ± 0	5 ± 1

[a]Table values are means ± standard error.

Table 3.3. Mesotrione metabolites at 3, 5, and 7 d after treatment (DAT) in mesotrione-tolerant (DKS35-70) and mesotrione-susceptible (84G62) grain sorghum[a].

		DKS35-70			84G62		
Compound	Retention Time	3 DAT	5 DAT	7 DAT	3 DAT	5 DAT	7 DAT
	min	% of radioactivity					
Metabolite 1	7	35 ± 2	29 ± 2	41 ± 3	28 ± 2	28 ± 1	43 ± 4
Metabolite 2	9.5	-	12 ± 1	12 ± 1	-	9 ± 3	14 ± 3
Metabolite 3	14	-	-	11 ± 9	-	-	-
Mesotrione	15.5	65 ± 2	59 ± 2	36 ± 8	72 ± 2	63 ± 2	43 ± 3

[a]Table values are means ± standard error.

Chapter 4 - Response of Acetyl Coenzyme A Carboxylase-Resistant Grain Sorghum to Quizalofop at Various Rates and Application Timings

Abstract

Conventional grain sorghum is highly susceptible to POST grass control herbicides. Development of acetyl-coenzyme A carboxylase-resistant grain sorghum could provide additional opportunities for POST herbicide grass control in grain sorghum. Field experiments were conducted at Hays and Manhattan, KS, to determine the effect of quizalofop rate and crop growth stage on injury and yield of acetyl-coenzyme A carboxylase-resistant grain sorghum. Quizalofop was applied at 62, 124, 186, and 248 g ai ha^{-1} at sorghum heights of 8 to 10, 15 to 25, and 30 to 38 cm, which corresponded to early POST (EPOST), mid-POST (MPOST), and late POST (LPOST) application timings, respectively. Grain sorghum injury ranged from 3 to 68% at 1 wk after treatment (WAT); by 4 WAT, plants generally recovered from injury. The EPOST and MPOST applications caused 9 to 68% and 2 to 48% injury, respectively, whereas injury from LPOST was 0 to 16%, depending on rate. Crop injury from quizalofop was more prominent at rates higher than the proposed use rate in grain sorghum of 62 g ha^{-1}. Sorghum grain yield was not affected by quizalofop as there were no significant differences in grain yield between herbicide-treated and non-treated plots regardless of rate or application timing.

Nomenclature: Quizalofop; sorghum, *Sorghum bicolor* (L.) Moench. SORBI.

Keywords: ACCase-inhibiting herbicides, growth stages, herbicide rate, crop response.

Introduction

In terms of acreage, grain sorghum is the third largest cereal crop grown in the United States (Anonymous 2010). Sorghum (*Sorghum bicolor* (L.) Moench) is grown mainly in dry, warm conditions, and encounters several weeds that grow faster than the crop and typically dominate resource utilization. The most common weed control problems in grain sorghum include grasses such as *Setaria*, *Eichinochloa, Digitaria, Panicum,* and *Sorghum* species (Robinson et al. 1964; Smith et al. 1990; Stahlman and Wicks; 2000). Norris (1980) reported that the presence of one barnyardgrass (*Eichinochloa crus-galli*) plant per meter of crop row reduced grain sorghum yields by nearly 10%, and 175 plants per meter-crop row reduced yield by 52%. Unless good weed control is achieved, substantial yield loss will occur. Weeds also decrease grain quality, increase insect and disease pressure, and increase harvest difficulty (Zimdahl 1999).

Crop rotation and tillage are often used to control grass weeds infesting grain sorghum; however, herbicides are still the major component of any sorghum weed control program (Brown et al. 2004). The main option for grass weed control in grain sorghum is PRE herbicides such as *S*-metolachlor, alachlor, and dimethenamid. However, grain sorghum is typically grown in dry conditions, and lack of soil moisture to activate PRE applications may decrease herbicide effectiveness. Controlling grass weeds that escape PRE control or germinate after grain sorghum has emerged is difficult because options for POST grass control are very limited. Currently, there are no POST herbicides that provide broad spectrum grass control for grain sorghum.

Acetyl coenzyme A carboxylase (ACCase)-inhibiting (group A/1) herbicides are commonly used to control grass weeds in many crops including soybean (*Glycine max*). The selectivity of these herbicides is based on their effects at the target site – the plastidic ACCase that catalyzes the first committed step in de novo fatty acid biosynthesis (Burton 1997; Gronwald 1994). These herbicides block fatty acid biosynthesis, which consequently alters the integrity of the cell membrane causing metabolite leakage and plant death (Devine and Shimaburuko, 1994). Group A/1 herbicides encompass three chemical families: phenylpyrazoline (DEN), cyclohexanediones (CHD), and aryloxyphenoxypropionates (APP). APP herbicides, such as quizalofop, are used as POST treatments to control grass weeds in soybeans, sunflower, cotton, and canola. Foliar-applied quizalofop effectively controlled wild oats (*Avena fatua*), green foxtail (*Setaria viridis*), yellow foxtail (*Seteria glauca*), barnyardgrass, and volunteer cereals (Parsells 1985). Unfortunately, POST application of quizalofop is not an option in conventional grain sorghum production because of the crop's high susceptibility to this herbicide. Recently, new options for POST weed control in grain sorghum have been developed by transferring a major ACCase resistance gene from a wild sorghum relative to elite grain sorghum (Tuinstra and Al-Khatib 2007). Resistance was caused by a tryptophan-to-cysteine mutation at location 2027 (Kershner et al. 2009). This mutation is known to provide resistance to APP but not CHD herbicides. Therefore, quizalofop has been selected to be registered for use on APP-resistant sorghum because of its high efficacy on weeds that are common in sorghum fields (http://ir4.rutgers.edu/FoodUse/food_Use2.cfm?PRnum=10092).

The introduction of this technology would allow more effective POST grass weed control in grain sorghum production. However, climatic variability along with crop and weed growth stages often require producers to be flexible in their herbicide options for weed control, which could include altering the time or rate of quizalofop application (Carter et al. 2007). Using the correct herbicide rate and application timing is very important to maximize weed control and minimize injury potential to crops. Although information is available on the effect of quizalofop application rates and timing on weed control, much less information is available on how the crop reacts to this herbicide. Therefore, the objective of this research was to determine the influence of quizalofop rate and application timing on APP-resistant grain sorghum response and grain yield.

Materials and Methods

Field experiments were conducted at the Kansas State University Ashland Bottom Research Field at Manhattan, KS (lat:39.12, long:-96.64) and Agricultural Research Center at Hays, KS (lat:38.85, long:-99.34) in 2009. Agronomic practices for grain sorghum production followed the Kansas State University Agricultural Experiment Station and Cooperative Extension Services recommendations (Regehr 1998). The soil at the Manhattan site was a Reading silt loam (fine-silty, mixed, superactive, mesic Pachic Argiudolls) with 3.7% organic matter and pH 6.3. The soil at the Hays site was a Crete silty clay loam (fine, smectitic, mesic Pachic Argiustolls) with 2.3% organic matter and pH 6.5. Planting dates were 21 May and 19 June in Hays and Manhattan locations, respectively.

A line of ACCase-resistant grain sorghum developed at Kansas State University was planted approximately 3 cm deep at 170,000 seeds/ha in rows spaced 76 cm apart. Plots were 3.1 m wide (4 rows) and 9.1 m long. The experimental design was a randomized complete block with a 3 × 5 factorial arrangement (3 application timings and 5 application rates). Treatments were replicated four times. Experimental plots were maintained free of weeds with a PRE application of *S*-metolachlor and atrazine at 1,410 and 1,120 g ai ha^{-1}, respectively, and removal by hand as needed. Quizalofop was applied POST at 62 (1x), 124 (2x), 186 (3x), and 248 (4x) g ai ha^{-1}. The 62 g ha^{-1} rate of quizalofop is the proposed field use rate for control of grass weeds (http://ir4.rutgers.edu/FoodUse/food_Use2.cfm?PRnum=10092). Treatments were applied with crop oil concentrate[1] at the rate of 1% v/v. A non-treated control was included for comparison in all application timings. Treatments were applied when grain sorghum was 8 to 10, 15 to 25, and 30 to 38 cm in height, which corresponded to early POST (EPOST), mid POST (MPOST), and late POST (LPOST) application timings, respectively. Quizalofop was applied with either a tractor-mounted sprayer or CO_2 pressurized backpack equipped with TT110015[2] nozzles calibrated to deliver 120 L ha^{-1} at 207 kPa or 140 L ha-^{1} at 221 kPa, respectively.

Grain sorghum was evaluated for herbicide injury at 1, 2 and 4 wk after treatment (WAT). Injury ratings were based on a scale of 0 to 100%, where 0 represents no injury 100 represents plant death. Data on plant height and days to half bloom were determined at flowering. Sorghum grain was mechanically harvested from the two middle rows of each plot and weighed, and grain yield was adjusted to 14% moisture content.

Sorghum injury and days to half bloom data at each rating time were subjected to regression analysis using SigmaPlot[3]. Slope of the regression was tested for significance using an F-test at $\alpha = 0.05$. Plant height and yield data were subjected to ANOVA using PROC MIXED in SAS[4] with quizalofop rate, application timing, and all possible interactions as fixed effects and replicates as random effects. In addition, orthogonal contrast ($P = 0.05$) was used to compare yields between mesotrione-treated and nontreated means. Means were compared using Fisher's Protected LSD test at $P \leq 0.05$. All data were checked for normality and homogeneity of variance.

Results and Discussion

Environmental Conditions

Monthly maximum and minimum temperatures were near the 30-year normal values from May to August and June to September for Hays and Manhattan sites, respectively. However, temperatures were slightly colder during the last two months of the growing season at both sites as compared to the 30-year normal (Figure 4.1). Total precipitation received from planting to harvesting ranged from 42 to 130 mm and 31 to 215 mm at Hays and Manhattan, respectively. At Hays, May to July were slightly drier months than the 30-year normal but was generally wetter from August to October. At Manhattan, it was usually wetter as compared to the 30-year normal except in September. In general, monthly maximum and minimum temperatures and total precipitation were near the 30-year normal values indicating that 2009 was a typical year for planting grain sorghum in both locations. The favorable conditions in both sites likely contributed greatly to the state record grain sorghum yields in 2009 (USDA 2009).

Grain sorghum injury

There were no significant differences in sorghum injury between locations at all timings; however, due to high initial injury data are presented for both locations (Figure 4.2).

Quizalofop caused injury symptoms to grain sorghum including chlorosis, necrosis, leaf distortion, stunting and slight purple leaf coloring; the latter was attributed to anthocyanin accumulation (Ishikawa et al. 1985; Swisher and Corbin 1982). Visual injury was first observed 5 to 7 d after treatment as irregular chlorotic areas on treated tissue that became progressively necrotic. Distorted leaf growth and subsequent stunting of the plant were observed 7 to 10 d after treatment. At lower rates (62 and 124 g ha^{-1}), initial injury symptoms were leaf chlorosis and slight leaf distortion. At the highest rate, especially when quizalofop was applied at EPOST, initial injury symptoms were severe chlorosis, stunting, and epinasty. Previous research also showed variability in grain sorghum injury related to mesotrione application rate and plant growth stage (Abit et al. 2010). Newly developing leaves were the first to show symptoms, followed by other developed leaves; however, all injury symptoms disappeared by the end of the growing season.

Quizalofop at all rates caused injury to grain sorghum in all application timings. Injury severity increased with increasing quizalofop rate, especially at the two earlier application timings. Quizalofop caused more injury at the EPOST and MPOST than at the LPOST timing 1 WAT at both sites (Figure 4.2). These results suggest that young, rapidly growing plants absorb more herbicide than mature plants, and are consistent with reports of others (Devine 1989; Wanamarta and Penner 1989). At 1 WAT, injury from EPOST application timing ranged from 6 to 13% when quizalofop was applied at 62 g ha^{-1} to 65 to 70% at the 248 g ha^{-1} rate. Injury ratings 2 WAT ranged from 4 to 60% when quizalofop was applied at 62 to 248 g ha^{-1}, respectively. At 4 WAT, plants generally recovered and produced normal shoots, except plants treated at 248 g ha^{-1} that showed less than 17% injury (data not shown). At MPOST quizalofop applied at 62 to 248 g ha^{-1} injured sorghum 2 to 48% at 1 WAT. However, by 2 WAT, injury dissipated except at the highest rate with less than 12% injury. Sorghum injury was less than 15% when quizalofop was applied LPOST at both sites. At 1 WAT, injury ranged from 2 to 16%. By 2 WAT, symptoms dissipated and new shoots appeared normal.

Maximum injury of 65 and 70% at Manhattan and Hays, respectively, occurred when quizalofop was applied at the highest rate, but plants were not killed. At 2 WAT, the proposed use rate of quizalofop (62 g ha^{-1}) generally did not visibly injure sorghum, the 124 g ha^{-1} rate caused slight necrosis and stunting, and the highest two rates of quizalofop caused moderate to severe necrosis and plant stunting. Data for sorghum injury at 4 WAT is not reported because no injury was observed for any treatment at that time except for the highest rate of quizalofop at the EPOST timing.

Agronomic Response

Plant height was similar when mesotrione was applied at all rates and application timings (data not shown). In addition, no treatment by site interaction was observed. Data on days to half bloom were averaged across locations because there was no location by rate by timing or location by treatment interactions that occurred. Sorghum flowering dates differed among application timings (Figure 4.3). No delay in flowering was observed when plants were treated at EPOST; however, there was a delay in flowering when quizalofop was applied at MPOST or LPOST, especially at the higher rates. Sorghum plants treated with 186 and 248 g ha^{-1} quizalofop at MPOST had a 4-d delay in flowering, whereas plants treated with 124, 186, and 248 g ha^{-1} quizalofop at LPOST had 5-, 6-, and 10-d delays in flowering, respectively. The flowering date at the LPOST herbicide application timing may be due to the lack of time for recovery before the plant initiates its reproductive phase (Smith et al. 2006). In areas where time of planting is not important, delayed flowering would not be much of a concern. However, in areas where time of planting is dictated by weather, such as Kansas, delay of flowering could likely impair harvest (Abit et al. 2010).

Grain yield

Significant interactions among application rates were not detected at either location; therefore, data for grain yield were pooled over rates. Although quizalofop caused significant injury, grain sorghum has shown the ability to recover from severe injury without sustaining yield reductions (Abit et al 2010). There were no differences in grain yield between treated and nontreated grain sorghum except at the MPOST and LPOST timings at Manhattan and Hays, respectively (Table 4.1). In both instances treated grain sorghum yielded more than non treated sorghum. Therefore, injury to APP-resistant grain sorghum from quizalofop did not negatively affect grain yield.

This study demonstrates that POST application of quizalofop could be applied at any growth stage because application timing is not critical and any injury to APP-resistant sorghum will not cause yield reduction. There is some level of resistance to quizalofop in this grain sorghum line; hence, it could provide flexibility in managing weeds in terms of application timing and rate.

Sources of Materials

[1]Prime Oil, Terra International Inc., P. O. Box 6000, Sioux City, IA 51102-6000.

[2]TeeJet, Spraying Systems Co., P. O. Box 7900, Wheaton, IL 60189-7900.

[3]Systat Software, Inc. 501 Canal Blvd, Suite E, Point Richmond, CA 94804-2028.

[4]SAS version 9.1, SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513.

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Figure 4.1. Monthly and 30-year maximum and minimum temperatures and total precipitation from planting to harvesting in Hays and Manhattan, 2009.

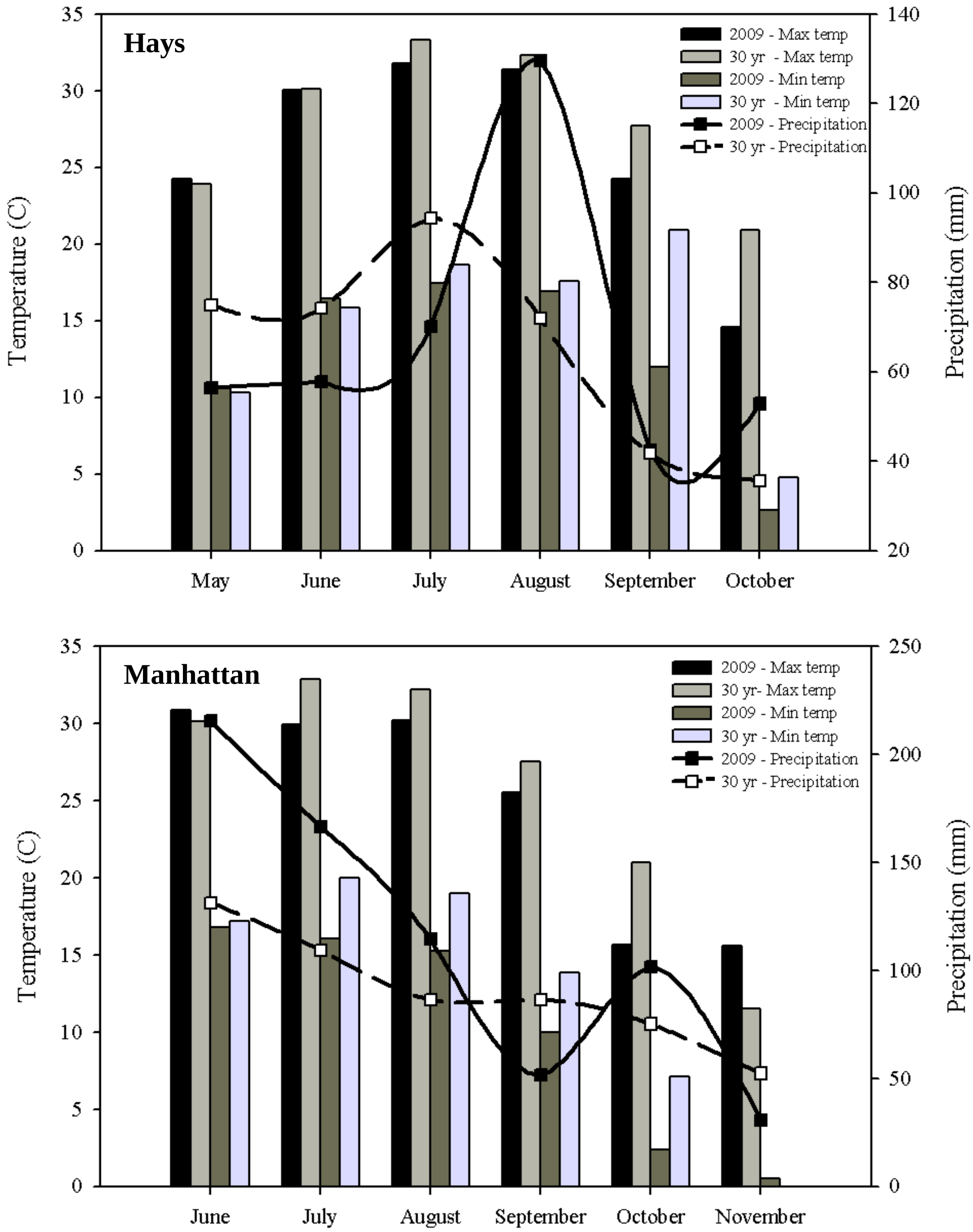

Figure 4.2. Quizalofop injury to ACCase-resistant grain sorghum at Hays and Manhattan sites as affected by quizalofop rate and timing 1 and 2 wk after treatment (WAT).

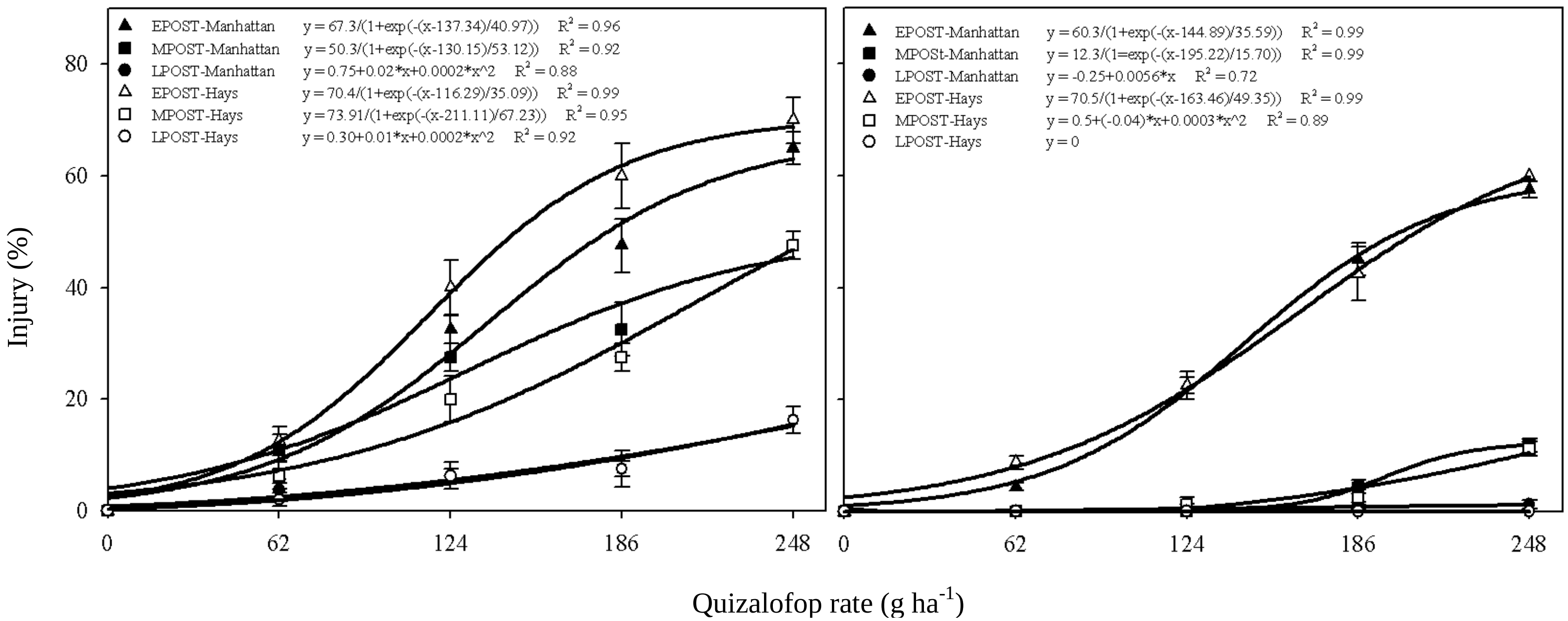

Figure 4.3. The effect of quizalofop rate and timing to days to half bloom of acetyl-coenzyme A carboxylase-resistant grain sorghum.

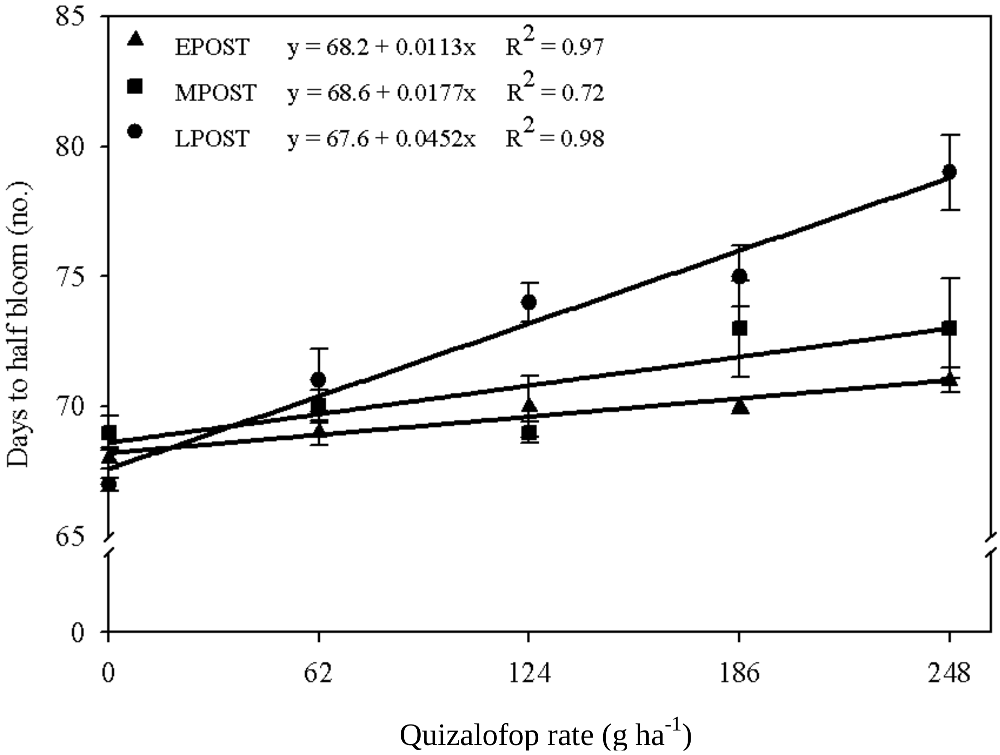

Table 4.1 Yield comparison of nontreated and quizalofop-treated ACCase-resistant grain sorghum as influenced by quizalofop application timing at Hays and Manhattan, KS.

Timing	Treatment	Yield[a] Hays	Manhattan
		kg ha^{-1}	
EPOST	Nontreated	2393	1886
	Treated	2853	1722
P-value		0.1509	0.3060
CV		11.4	13.3
MPOST	Nontreated	2156	1618
	Treated	2655	1874
P-value		0.1383	0.0060
CV		11.9	12.1
LPOST	Nontreated	1826	1702
	Treated	2592	1735
P-value		0.0097	0.8267
CV		11.8	13.1

[a]Averaged across quizalofop

Chapter 5 - Efficacy of Postemergence Herbicide Tankmixes in Acetyl Coenzyme A Carboxylase Resistant Grain Sorghum

Abstract

The development of acetyl coenzyme A carboxylase (ACCase)-resistant grain sorghum could provide additional opportunities for POST herbicide grass control in grain sorghum. Field experiments were conducted in Kansas (Dodge City, Garden City, Hays, Manhattan, Colby, Ottawa, and Tribune), South Dakota (Highmore), and Texas (Bushland, and Yoakum) to evaluate the efficacy of quizalofop tank mixes in ACCase-resistant grain sorghum. Quizalofop was applied alone or in combination with dicamba, 2,4-D, prosulfuron, 2,4-D + metsulfuron methyl, or halosulfuron + dicamba. Herbicides were applied when sorghum was 12 to 50 cm in height. Overall weed control was greater when quizalofop was applied with other herbicides than when applied alone. At 2 and 4 weeks after treatment (WAT), grass weed control was greater than 90% and 80%, respectively, when quizalofop was applied alone or in combination with dicamba, halosulfuron + dicamba, or prosulfuron. Broadleaf weed control was greater than 90% in all treatments except when quizalofop was applied alone. Herbicide treatments except those that included 2,4-D caused slight to no sorghum injury. Grain sorghum yield was greater for all herbicide treatments compared to the weedy check. This research showed that application of quizalofop in combination with broadleaf weed control herbicides provided excellent weed control in sorghum.

Nomenclature: Quizalofop; dicamba; 2,4-D; prosulfuron; metsulfuron methyl; halosulfuron; atrazine; *S*-metolachlor; sorghum, *Sorghum bicolor* (L.) Moench. SORBI.
Keywords: ACCase-inhibiting herbicides, herbicide resistant crop.

Introduction

Grain sorghum is one of the major cereal crops grown in the United States. This crop is generally cultivated in areas that are too hot or dry for successful corn production (Bennett et al. 1990). Grain sorghum is used primarily as an animal feed, but is also used in food products, as an industrial feedstock, and for biofuels. Sorghum development and grain yield are influenced by numerous abiotic and biotic factors, including weeds. Historically, broadleaf species were the predominant weeds in grain sorghum; but annual grass species are increasing in importance in some production areas. Crabgrass spp., shattercane, johnsongrass, foxtail spp., kochia, cocklebur, and pigweeds are among the most common weeds in grain sorghum in the U.S (Stahlman and Wicks 2000; Bridges 1992). Uncontrolled weeds typically reduce sorghum grain yield 30 to 50% but losses can be much higher under extreme conditions (Stahlman and Wicks 2000). Wiese et al. (1983) reported yield loss of 48% in grain sorghum fields infested with either johnsongrass or shattercane. Others have reported 40 to >50% reductions in sorghum yield with 1 to 12 redroot pigweed plants per meter of row (Knezevic et al. 1997; Phillips 1960).

Producers primarily rely on herbicides to control weeds in sorghum, with 85% of the sorghum planted in the United States receiving some type of herbicide treatment

(USDA 2004). The main option for grass weed control in grain sorghum is use of a PRE herbicide such as *S*-metolachlor, alachlor, or dimethenamid. However, the efficacy of PRE herbicides is moisture dependent. Too little or excessive moisture after application can result in less than optimum weed control (Tapia et al 1997). In general, controlling grass weeds that emerge after crop establishment is difficult because options for POST grass control are very limited. Currently, there is no single herbicide option available for POST control of grass weeds for grain sorghum.

Quizalofop, an aryloxyphenoxypropionate (APP) herbicide, is a selective POST graminicide that effectively controls annual and perennial grasses. It inhibits the chloroplastic acetyl coenzyme A carboxylase (ACCase) and disrupts fatty acid biosynthesis in susceptible plants (Gronwald 1991). Unfortunately, quizalofop is not an option on conventional grain sorghum because of the crop's susceptibility to this herbicides. The development of ACCase-resistant grain sorghum could allow the use of quizalofop for grass control in grain sorghum. Recently, ACCase-resistant grain sorghum was developed by transferring a major ACCase resistance gene from a wild sorghum relative to elite grain sorghum (Tuinstra and Al-Khatib 2007). Resistance was caused by a tryptophan-to-cysteine mutation at location 2027 (Kershner et al. 2009). This mutation is known to provide resistance to APP but not cyclohaxanedione herbicides. Therefore, quizalofop has been selected to be registered for use on APP-resistant sorghum because of its high efficacy on weeds that are common in sorghum fields (http://ir4.rutgers.edu/FoodUse/ food_Use2.cfm?PRnum=10092).

Although annual and perennial grass weeds can be controlled in ACCase-resistant grain sorghum with quizalofop, control of broadleaf weeds requires that additional herbicides be tank mixed with quizalofop. Tank mixing to control broadleaf and grass weed species is a common practice that is increasingly used in most agronomic crops to save time and reduce application costs, and/or prevent the development of herbicide-resistant weeds (Zhang et al. 1995; Hatzios and Penner 1985). However, combinations of APP herbicides with herbicides used to control broadleaf weeds typically result in antagonistic reactions (Barnes and Oliver 2004; Gerwick et al. 1988; Minton et al. 1989; Virdine 1989; Olson and Nalewaja 1981). Developing weed management systems requires an understanding of how herbicides react when mixed together. The objective of these studies was to evaluate the efficacy of quizalofop tank mixes in ACCase-resistant grain sorghum.

Materials and Methods

Field experiments were conducted at Kansas (Dodge City, Garden City, Hays, Manhattan, Colby, Ottawa, and Tribune), South Dakota (Highmore), and Texas (Bushland, and Yoakum) in 2009. Agronomic practices for grain sorghum production were typical for the area. Geographical location, soil type, soil taxonomic class, percentage organic matter, and soil pH are shown in Table 5.1. A genetic line of ACCase-resistant grain sorghum was planted approximately 3 cm deep at 87,500 to 172,500 seeds/ha (Table 5.1) in rows spaced 76 cm apart. Plots were 3 m wide to accommodate four rows and 9.1 m in length. Natural populations of weed species in each site are presented in Tables 5.3 and 5.4. Herbicides were applied using a CO_2-pressurized backpack or a tractor-mounted sprayer equipped with either TeeJet[1] XR8002, XR11002, TT11003, TT11004, or TT110015 flat fan nozzles, calibrated to deliver 187 L ha^{-1} at a pressure of 138 to 252 kPa.

Herbicides treatments were POST application of quizalofop at 62 g ai ha^{-1} alone and in combination with dicamba, 2,4-D, prosulfuron, 2,4-D + metsulfuron methyl, or halosulfuron + dicamba at rates of 281, 280, 20, 140 + 2, and 39 + 140 g ai ha^{-1}, respectively. A non-treated control and standard PRE treatment of *S*-metolachlor + atrazine at 1076 + 1390 g ai h^{-1} were included for comparison. POST herbicide treatments were applied when sorghum was 12 to 50 cm in height (Table 5.2). All herbicides treatments except *S*-metolachlor + atrazine included PRE application of atrazine at 2.2 kg ha^{-1}, 1% vol/vol crop oil concentrate[2] (except herbicide treatments with 2,4-D), and 2.2 kg ha^{-1} ammonium sulfate.

Sorghum injury and broadleaf and grass weed control were estimated by visual ratings on a scale of 0 (no injury/control) to 100% (crop death/complete control) at 2 and 4 wk after POST treatments. Sorghum grain was mechanically harvested from the middle two rows of each plot, weighed, and grain yield was adjusted to 14% moisture content.

The experiment design was a randomized complete block with treatments replicated four times. Data were tested for homogeneity of variances and normality of distribution and were square root transformed as needed before analysis of variance. All data were subjected to ANOVA and analyzed by SAS[3] PROC MIXED with herbicide treatments and location as a fixed effects, and replicates and replicate(location) as random effects. The nontreated check was excluded from the ANOVA. Treatment means were separated by Fisher's Protected LSD text at $P \leq 0.05$. Data are presented as untransformed means.

Results and Discussion

Site by treatment interactions prevented the pooling of data; therefore, data are presented by site and treatment for sorghum injury and weed control ratings. At 2 WAT, slight to no sorghum injury was observed from treatments except those that included 2,4-D (Table 5.5). Most injury consisted of bleaching, however those treatments containing 2,4-D also exhibited epinasty. The highest amount of injury was from those treatments that caused both epinasty and bleaching. Injury ratings at 4 WAT were considerably less severe compared to ratings at 2 WAT, indicating sorghum recovery. However, injury was still evident for treatments containing 2,4-D. Leaf malformation was still visible on lower leaves while new growth appeared unaffected. Crop injury from other POST herbicide treatments had dissipated and growth appeared normal. Hays injury data was not included in the analysis due to tank contamination during spraying.

A total of five different grass weed species were rated; with three species at Garden City, four species at Manhattan and one species each at Dodge City, Hays, Ottawa, Highmore and Bushland (Table 5.3). Giant foxtail (*Setaria faberi* Herm.) and large crabgrass (*Digitaria sanguinalis* (L.) Scop.) were the most frequent species in all of the sites. No grass weed species were present at Colby, Tribune, or Bushland. Overall grass control from POST herbicide treatments varied among sites (Table 5.6). Grass control at 2 WAT with quizalofop or in tank mixtures with dicamba, halosulfuron + dicamba, or prosulfuron was 90% or greater except at the Hays, Ottawa and Yoakum sites. Less grass control in Hays, Ottawa, and Yoakum sites was due to the presence of very dense population of green foxtail, giant foxtail, and Texas panicum in these respective sites. Tank mixing with 2,4-D or 2,4-D + metsulfuron methyl with quizalofop resulted in a 0 to 12 and 1 to 26% reduction in grass control respectively compared to quizalofop alone. At 4 WAT, grass control increased in all POST herbicide treatments except when quizalofop was applied with 2,4-D, which was reduced by 5 to 17% (Table 5.6). Similar results were observed with other aryloxyphenoxypropionic herbicides when applied in mixtures with auxin-type broadleaf weed herbicides (Blackshaw et al. 2006; Olson and Nalewaja 1981; Shimabukuro et al. 1986; Barnwell and Cobb 1993). The observed results could be due to the antagonism of quizalofop by 2,4-D. It is likely that the presence of 2,4-D decreased the conversion of quizalofop from the quizalofop-ethyl to the active acid form, decreased translocation of quizalofop, increased the rate of detoxification, and competed at the fatty acid synthesis level (Tu et al. 2001).

A total of twelve broadleaf weed species were rates in all experimental sites. Palmer amaranth (*Amaranthus palmeri* S. Wats.) and puncturevine (*Tribulus terrestris* L.) were the most common species observed. No broadleaf weed species were observed in Colby site, KS (Table 5.4). Broadleaf weed control at the Yoakum site was not included in the analysis due to unexplained inconsistencies. Broadleaf weed control was greater when quizalofop was applied with various broadleaf herbicides than when applied alone (Table 5.7). Overall broadleaf control was greater than 90% for all POST herbicide treatments 2 WAT, except when quizalofop was applied alone. Control of weeds in plots treated with quizalofop alone was due to the PRE application of atrazine. All herbicide treatment combinations provided excellent broadleaf weed control than the check or standard treatments. It is interesting to note that broadleaf weed control was still excellent

4 WAT with all POST herbicide combinations, although control in plots treated with quizalofop + 2,4-D decreased by 1 to 13%. The continued control was probably due to the residual activity of the tank mixed broadleaf herbicides that provided a sufficient degree of control of later germinating weeds.

Crop yields were determined only at Hays, Manhattan, and Tribune sites. Significant interactions between locations for grain yield data were not detected; therefore, data were pooled over locations. Grain yield was greater in plots treated with herbicides than in the nontreated weedy check. The highest yields were in plots treated with quizalofop + prosulfuron, quizalofop + halosulfuron + dicamba, quizalofop + dicamba at 2,505, 2486, and 2,376 kg ha^{-1}, respectively (Table 5.8). There was a well correlation between grass and broadleaf control and sorghum grain yield (r = 0.71 and 0.55, respectively) when data was pooled across grass and broadleaf weed control 2 WAT.

The study showed that application of quizalofop in combination with broadleaf herbicides provided excellent weed control in the new ACCase-resistant grain sorghum. The increase in weed control resulted in significant increases in grain sorghum yields. To maximize weed control, quizalofop needs to be tank mixed with other broadleaf herbicides. However, tank mixing 2,4-D with quizalofop may decrease control of grass weed species.

Sources of Materials

[1] TeeJet Spraying Systems, Wheaton, IL 60189-7900.

[2] Prime Oil, Terra International Inc., P.O. Box 6000, Sioux City, IA 51102-6000.

[3] SAS version 9.1, SAS Institute Inc., 100 SAS Campus Drive, Cary, NC 27513.

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Table 5.1. Geographic location, soil characteristics, seeding dates and rates for eight experimental sites in 2009.

						Seeding	
Site	Geographic location	Soil type	Soil taxonomic class	% OM	Soil pH	Date	Rate
							seeds/ha
Colby	Northwest Kansas	Keith silt loam	Aridic Agriustolls	2.3	6.2	June 6	86,500
Dodge City	Southwest Kansas	Harney silt loam	Typic Agriustolls	-	-	June 8	105,000
Garden City	Southwest Kansas	Ulysses silt loam	Aridic Haplustolls	1.4	8.0	June 25	100,000
Hays	West-Central Kansas	Crete silty clay loam	Pachic Agriustolls	2.3	6.5	May 21	172,500
Manhattan	Northeast Kansas	Smolan silty clay loam	Pachic Agriustolls	4.3	5.8	June 19	135,000
Ottawa	East-Central Kansas	Woodson silt loam	Abruptic Argiaquolls	3.0	6.7	June 24	172,500
Tribune	Southwest Kansas	Ulysses silt loam	Aridic Haplustolls	2.0	8.3	June 8	102,500
Bushland	Texas Panhandle	Pullman silty clay loam	Torrertic Paleustolls	1.3	7.6	June 10	112,500
Yoakum	Southeast Texas	Denhawken sandy loam	Vertic Haplustepts	1.0	7.6	May 6	109,500
Highmore	Central South Dakota	Glenham loam	Typic Agriustolls	2.1	6.4	May 27	172,500

- = No data available

Table 5.2. Herbicide PRE and POST application dates and grain sorghum height at POST application for eight experimental sites in 2009.

Site	Geographic location	Application date		Crop height at POST application
		PRE	POST	
				cm
Colby	Northwest Kansas	May 31	July 10	25 to 35
Dodge City	Southwest Kansas	June 9	June 30	15 to 30
Garden City	Southwest Kansas	June 25	August 4	15 to 30
Hays	West-Central Kansas	May 22	June 29	15 to 35
Manhattan	Northeast Kansas	June 20	July 17	20 to 35
Ottawa	East-Central Kansas	June 24	July 22	20 to 35
Tribune	Southwest Kansas	June 8	June 30	12 to 20
Bushland	Texas Panhandle	June 11	July 7	15 to 30
Yoakum	Southeast Texas	May 7	June 8	40 to 50
Highmore	Central South Dakota	May 27	June 22	20 to 35

Table 5.3. Predominant grass weed species at each experimental location in 2009.

Site	Weed species	Scientific name	Bayer code
Colby, KS	None		
Dodge City, KS	Large crabgrass	*Digitaria sanguinalis* (L.) Scop.	DIGSA
Garden City, KS	Giant foxtail	*Setaria faberi* Herrm.	SETFA
	Large crabgrass	*Digitaria sanguinalis* (L.) Scop.	DIGSA
	Longspine sandbur	*Cenchrus longispinus* (Hack.) Fern.	CCHPA
Hays, KS	Green foxtail	*Setaria viridis* (L.) Beauv	SETVI
Manhattan, KS	Giant foxtail	*Setaria faberi* Herrm.	SETFA
	Green foxtail	*Setaria viridis* (L.) Beauv	SETVI
	Large crabgrass	*Digitaria sanguinalis* (L.) Scop.	DIGSA
	Barnyardgrass	*Echinochloa crus-galli* (L.) Beauv.	ECHCG
Ottawa, KS	Giant foxtail	*Setaria faberi* Herrm.	SETFA
Tribune, KS	None		
Highmore, SD	Green foxtail	*Setaria viridis* (L.) Beauv	SETVI
Bushland, TX	None		
Yoakum, TX	Texas panicum	*Panicum texanum* Buckl.	PANTE

Table 5.4. Predominant broadleaf weed species at each experimental location in 2009.

Site	Weed species	Scientific name	Bayer code
Colby, KS	None		
Dodge City, KS	Tumble pigweed	*Amaranthus albus* L.	AMAAL
Garden City, KS	Palmer amaranth	*Amaranthus palmeri* S. Wats.	AMAPA
	Kochia	*Kochia scoparia* (L.) Schrad.	KCHSC
	Russian thistle	*Salsola tragus* L.	SASKR
	Puncturevine	*Tribulus terrestris* L.	TRBTE
	Velvetleaf	*Abutilon theophrasti* Medik.	ABUTH
Hays, KS	Puncturevine	*Tribulus terrestris* L.	TRBTT
Manhattan, KS	Palmer amaranth	*Amaranthus palmeri* S. Wats.	AMAPA
	Common waterhemp	*Amaranthus rudis* Sauer Beauv.	AMATA
Ottawa, KS	None		
Tribune, KS	Puncturevine	*Tribulus terrestris* L.	TRBTE
	Pigweed spp.		
Highmore, SD	Wild buckwheat	*Polygonum convolvulus* L.	POLCO
	Prostrate pigweed	*Amaranthus blitoides* S. Wats.	AMABL
Bushland, TX	Palmer amaranth	*Amaranthus palmeri* S. Wats.	AMAPA
Yoakum, TX	Smellmellon	*Cucumis melo* L.	CUMMD

Table 5.5. Grain sorghum injury 2 and 4 wk after treatment as affected by quizalofop applied alone or in combination with selected herbicides.

		2 WAT							4 WAT						
Treatments	Rate	Bushland	Colby	Dodge City	Manhattan	Ottawa	Tribune	Yoakum	Bushland	Colby	Dodge City	Manhattan	Ottawa	Tribune	Yoakum
	g ha^{-1}	%													
Atrazine fb quizalofop + dicamba	62 + 281	28	3	14	1	4	10	0	14	3	10	0	0	4	0
Atrazine fb quizalofop + 2,4-D + metsulfuron methyl	62 + 140 + 2	31	10	33	4	70	55	5	19	3	36	0	24	60	0
Atrazine fb quizalofop + halosulfuron + dicamba	62 + 39 + 140	21	4	1	0	6	10	0	6	0	1	0	1	9	0
Atrazine fb quizalofop + 2,4-D	62 + 20	39	11	24	0	6	24	3	45	6	25	0	0	9	0
Atrazine fb quizalofop + prosulfuron	62 + 280	8	4	0	3	6	0	1	3	0	0	0	3	0	0
Atrazine fb quizalofop	62	0	0	0	0	1	3	3	0	0	0	0	0	0	0
S-metolachlor + atrazine	1076 + 1390	8	1	3	0	0	0	0	3	0	0	0	1	0	0
LSD (0.05)		12	3	14	NS	4	17	NS	11	5	10	NS	4	8	NS

All herbicide treatments except *S*-metolachlor + atrazine included 1% v/v crop oil concentrate, and 2.2 kg ha^{-1} ammonium sulfate.

Table 5.6. Grass weed control 2 and 4 wk after treatment as affected by quizalofop applied alone or in combination with selected herbicides

		2 WAT							4 WAT						
Treatments	Rate	Dodge City	Garden City	Hays	Manhattan	Ottawa	Highmore	Yoakum	Dodge City	Garden City	Hays	Manhattan	Ottawa	Highmore	Yoakum
	g ha^{-1}	%													
Atrazine fb quizalofop + dicamba	62 + 281	96	98	68	92	91	95	88	98	-	69	92	92	99	84
Atrazine fb quizalofop + 2,4-D + metsulfuron methyl	62 + 140 + 2	84	89	73	70	79	97	84	89	-	71	82	81	99	66
Atrazine fb quizalofop + halosulfuron + dicamba	62 + 39 + 140	94	99	63	90	76	94	78	93	-	68	90	88	99	82
Atrazine fb quizalofop + 2,4-D	62 + 20	95	93	80	87	88	98	77	89	-	70	70	85	99	72
Atrazine fb quizalofop + prosulfuron	62 + 280	100	96	80	97	86	98	93	98	-	85	97	82	99	90
Atrazine fb quizalofop	62	100	99	80	96	88	98	89	100	-	84	96	93	95	84
S-metolachlor + atrazine	1076 + 1390	96	93	90	98	68	82	18	94	-	90	98	68	99	40
LSD (0.05)		14	6	5	7	14	7	24	9	-	6	6	22	NS	22

All herbicide treatments except *S*-metolachlor + atrazine included 1% v/v crop oil concentrate, and 2.2 kg ha^{-1} ammonium sulfate.

\- = No data available

Table 5.7. Broadleaf weed control 2 and 4 wk after treatment as affected by quizalofop applied alone or in combination with selected herbicides.

		2 WAT							4 WAT						
Treatments	Rate	Bushland	Dodge City	Garden City	Hays	Manhattan	Highmore	Tribune	Bushland	Dodge City	Garden City	Hays	Manhattan	Highmore	Tribune
	g ha^{-1}							%							
Atrazine fb quizalofop + dicamba	62 + 281	94	98	99	96	93	98	96	96	97	-	99	86	93	93
Atrazine fb quizalofop + 2,4-D + metsulfuron methyl	62 + 140 + 2	100	100	99	96	94	99	100	100	100	-	100	91	99	99
Atrazine fb quizalofop + halosulfuron + dicamba	62 + 39 + 140	96	100	98	94	95	92	100	97	100	-	100	88	97	97
Atrazine fb quizalofop + 2,4-D	62 + 20	96	100	99	97	98	98	92	95	95	-	94	85	86	86
Atrazine fb quizalofop + prosulfuron	62 + 280	96	100	96	95	96	99	97	91	100	-	97	94	100	100
Atrazine fb quizalofop	62	80	98	97	82	87	94	81	75	95	-	81	88	71	71
S-metolachlor + atrazine	1076 + 1390	99	100	96	82	84	85	94	96	99	-	77	44	91	91
LSD (0.05)		12	NS	2	3	3	10	8	23	7	-	4	27	18	18

All herbicide treatments except *S*-metolachlor + atrazine included 1% v/v crop oil concentrate, and 2.2 kg ha^{-1} ammonium sulfate.

- = No data available

Table 5.8. Grain sorghum yield as affected by quizalofop applied alone or in combination with selected herbicides.

Treatments	Rate	Grain Yield
	g ha^{-1}	kg ha^{-1}
Atrazine fb quizalofop + dicamba	62 + 281	2376
Atrazine fb quizalofop + 2,4-D + metsulfuron methyl	62 + 140 + 2	2042
Atrazine fb quizalofop + halosulfuron + dicamba	62 + 39 + 140	2486
Atrazine fb quizalofop + 2,4-D	62 + 20	2072
Atrazine fb quizalofop + prosulfuron	62 + 280	2505
Atrazine fb quizalofop	62	2198
S-metolachlor + atrazine	1076 + 1390	2399
Weedy check		1441
LSD (0.05)		559

All herbicide treatments except *S*-metolachlor + atrazine included 1% v/v crop oil concentrate, and 2.2 kg ha^{-1} ammonium sulfate.

Chapter 6 - Metabolism of Quizalofop and Rimsulfuron in Herbicide Resistant Grain Sorghum

Abstract

Studies were conducted to determine if herbicide metabolism is a mechanism that could explain the resistance of ACCase- and ALS-resistant grain sorghum to quizalofop and rimsulfuron, respectively. ACCase- and ALS-resistant and -susceptible genetic lines were grown under controlled conditions and treated at the 4-leaf stage with ^{14}C-labeled quizalofop and rimsulfuron on separate. Plants were harvested at 3, 5, and 7 d after treatment. In the ACCase metabolism experiment, resistant grain sorghum transformed 88% of inactive quizalofop-ethyl to active quizalofop while 91% of the inactive was converted to active form by the susceptible plants 3 DAT. By 7 DAT, all inactive quizalofop-ethyl was converted to active quizalofop. In the ALS metabolism study, two distinct metabolites were produced from rimsulfuron. Metabolism rate was similar between resistant lines (TX430R and N223R) in all harvest dates except at 7 DAT; however, metabolism was more rapid in the resistant lines than in the susceptible genotypes (TX430S and N223S). The percentage of recovered rimsulfuron 3 DAT corresponded to 80 and 83% in the resistant compared to 87% in the susceptible grain sorghum. At 5 DAT, metabolism was near steady in all sorghum plants but by 7 DAT, resistant genotypes metabolized 4 to 12% more than the susceptible sorghum. Rapid metabolism of rimsulfuron in ALS-resistant grain sorghum is an added mechanism that could help evaluate the level of rimsulfuron resistance.

Nomenclature: Quizalofop; rimsulfuron; sorghum, *Sorghum bicolor* (L.) Moench. SORBI.

Keywords: ACCase-inhibiting herbicides; ALS-inhibiting herbicides; herbicide resistant crop.

Introduction

Preemergence (PRE) herbicide programs have been the mainstay for grass weeds in grain sorghum. However, grain sorghum is typically grown in dry conditions, and lack of soil moisture to activate PRE applications may decrease herbicide performances (Tapia et al 1997). Producers with fields that have heavy grass pressure prefer to plant crops other than sorghum because there is no effective herbicide option available for POST control of grass weeds for grain sorghum.

Quizalofop and rimsulfuron are postemergence (POST) herbicides that effectively control grass weeds. Quizalofop, an acetyl-coenzyme A carboxylase- (ACCase) inhibiting herbicide belonging to the aryloxyphenoxypropionates (APP) herbicide family, is commonly used to control grass weeds in many crops including soybean (*Glycine max*), cotton (*Gossypium hirsutum*), sunflower (*Helianthus annuus*), and canola (*Brassica napus*). ACCase is a multifunctional enzyme that catalyzes the synthesis of malonyl-CoA in the first committed step of the *de novo* fatty acid biosynthesis (Harwood, 1988; Schmid et al., 1997). ACCase-inhibiting herbicides block the action of the ACCase preventing fatty acid biosynthesis (Devine and Shimaburuko, 1994). Foliar-applied quizalofop effectively controlled several common grasses in sorghum fields such as green foxtail (*Setaria viridis*), yellow foxtail (*Seteria glauca*), barnyardgrass (*Echinochloa crus-galli*), and johnsongrass (Parsells 1985).

Rimsulfuron is a sulfonylurea herbicide that inhibits acetohydroxyacid synthase, also known as acetolactate synthase (ALS), which is the first enzyme unique to biosynthesis of the essential branched-chain amino acids leucine, valine, and isoleucine (Babczinski and Zelinski 1991; Ray 1984). The enzyme can either catalyze formation of acetohydroxybutyrate from pyruvate and α-ketobutyrate or synthesis of acetolactate from two molecules of pyruvate (Umbarger 1969). Rimsulfuron provides more than 95% control of johnsongrass (Damalas and Eleftherohorinos 2001). In addition, rimsulfuron controls several troublesome grass weeds in sorghum fields such as proso millet (*Panicum miliaceum* L.), green foxtail, and giant foxtail (*Setaria faberi* Herrm) (Mekki and Leroux 1994).

A major limitation for usage of quizalofop and rimsulfuron in grain sorghum is the high susceptibility of the crop to these herbicides. Grain sorghum resistance to quizalofop and rimsulfuron, however, has been developed by transferring ACCase and ALS resistance genes from a wild sorghum relative to elite grain sorghum (Tuinstra and Al-Khatib 2007). Resistance in ACCase was caused by a tryptophan-to-cysteine mutation at location 2027 (Kershner et al. 2009). This mutation is known to provide resistance to aryloxyphenoxy- propionates (APP) but not cyclohexanediones herbicides. Conversely, resistance in ALS was due to tryptophan-574-leucine mutation (Kershner 2010). Leucine-574 is a mutation that provides strong cross resistance to imidazolinone and sulfonylurea herbicides.

Resistance to herbicides could be due to several mechanisms. In ACCase-inhibiting herbicides, resistance could occur by one or more of three possible mechanisms (Délye, 2005; DePrado et al. 2000; Gronwald et al. 1992; Kershner 2010). These mechanisms include presence of less sensitive form of ACCase (alteration of target site enzyme), overproduction of ACCase, or metabolism-based detoxification of the herbicide. In ALS-inhibitors, resistance could be due to less herbicide sensitive ALS enzyme (altered site of action), or rapid metabolic inactivation of herbicide, or both (Cotterman and Saari 1992; Neighbors and Privalle 1990; Saari et al. 1990; Tranel and Wright 2002).

Previous research has shown that alteration of the ACCase and ALS enzyme confers resistance in grain sorghum (Kershner 2010). However, ALS- and ACCase-resistant grain sorghum plants expressed slight rimsulfuron and quizalofop injury symptoms 7 d after treatments (DAT). These symptoms usually dissipated 14 to 21 DAT (Abit et al. *unpublished data*; Hennigh 2010). The recovery from quizalofop and rimsulfuron injury may suggest that plants metabolized the herbicides. Thus, investigation on other mechanisms such as metabolism-based detoxification of ACCase- and ALS-inhibiting herbicides can provide additional insight on the mechanism of resistance in the newly developed crops.

The objective of this research was to determine if a change in rate of metabolism is an additional mechanism that could explain the resistance of ACCase- and ALS-resistant grain sorghum to quizalofop and rimsulfuron, respectively.

Materials and Methods

Plant materials and growth conditions

In the ACCase experiment, ACCase-resistant and -susceptible grain sorghum genotypes were planted in separate 11-cm diameter containers filled with sand:Morill loam (fine-loamy, mixed, mesic Typic Argiudolls) soil (1:1 by vol) with pH 7.3 and 1.1% organic matter. Plants were grown under growth chamber conditions with 30/25 ± 2 C day/night temperatures, 16-h photoperiod, with supplemental light intensity of 250 ± 30 µmol m^{-2} s^{-1} photosynthetic photon flux. Plants were watered daily and fertilized once before treatment with commercial fertilizer solution[1] containing 1.2 g L^{-1} total nitrogen, 0.4 g L^{-1} phosphorus, and 0.8 g L^{-1} potassium.

In the ALS study, ALS-resistant ('TX430R' and 'N223R') and ALS-susceptible ('TX430S' and 'N223S') grain sorghum lines were grown under similar conditions as described in the ACCase study. All resistant lines (ACCase and ALS) were developed by Tuinstra and Al-Khatib (2003).

Radiolabelling experiments

ACCase experiment. At the 4-leaf stage, ACCase-resistant and -susceptible grain sorghum genotypes were treated with 10, 1 µl droplets (five droplets either side of the midrib) of ^{14}C-labeled quizalofop with specific activity of 4.25 MBq mg^{-1}, on the adaxial surface of the third leaf. A single 1-µl droplet contained 402 Bq of ^{14}C-quizalofop. Unlabeled quizalofop was added to the radioactive solution to obtain 62 g ai ha^{-1}, in a carrier volume of 187 L^{-1}. Crop oil concentrate[2] was also added at 1% v/v to enhance droplet-to-leaf surface contact.

Treated leaves were harvested at 3, 5, and 7 d after treatment (DAT). An acetone (2 ml of acetone/g of tissue) was used to remove unabsorbed ^{14}C quizalofop (Koeppe et al. 1990). Plant tissues were then frozen with liquid nitrogen, and ground with a mortar and pestle. In order to confirm radioactive herbicides were absorbed by the plants and to know the level of radioactivity in the ground samples, subsamples of the plants were weighed and oxidized. Captured $^{14}CO_2$ was measured using the liquid scintillation spectrometry (LSS)[3]. Leaf tissues were stored at -80 C until radioactivity was extracted.

Frozen tissues were homogenized with methylene chloride-acetone (1:1, v/v) (2 ml of solvent/g of tissue). The tissue-solvent mixture was shaken for 1 h, centrifuged at 714 g for 15 min, and decanted through Whatman No. 4 filter paper[4] (Koeppe et al. 1990). Quizalofop in the tissues were extracted and filtered two more times with methylene chloride-acetone using the same procedure. The three supernatant were pooled and were evaporated at 35 C to 0.5 ml using a centrivap[5]. Solution were filtered with a 0.2 filter paper[6] and stored at -20 C until use. To determine the amount of radioactivity not extracted into the supernatant, the remaining plant residue and filter paper were oxidized, and radioactivity was measured.

ALS experiment. Ten 1-µl droplets containing 3,453 Bq of ^{14}C-rimsulfuron were applied as described in the ACCase experiment to ALS-resistant and -susceptible genotypes. Unlabeled rimsulfuron was added to the radioactive solution to obtain 18 g ai ha^{-1}, in a carrier volume of 187 L^{-1}. Nonionic surfactant[7] was also added at 0.25% v/v to enhance droplet-to-leaf surface contact. At 3, 5, and 7 DAT, the treated leaves were excised and washed with 15 ml of 75% methanol, then frozen with liquid nitrogen, and ground with mortar and pestle (Schuster 2007). Subsamples and radioactivity of subsamples were determined by using LSS. Leaf tissues were stored at -20 C until radioactivity was extracted.

Frozen tissues were homogenized using 15 ml of 75% methanol (by vol) and shaken for 1 h. Samples were filtered with Whatman 4 filter paper and the supernatant was saved. The remaining leaf tissues were resuspended in 5 ml of 90% methanol and shaken for an additional hour. Samples were filtered, and the supernatant was added to the first supernatant then total supernatants was evaporated, filtered, and stored using the same procedure as describe above. Amount of radioactivity not extracted into the supernatant was also determined.

High performance liquid chromatography (HPLC) analysis

Extracts from the ACCase and ALS experiments were injected into a Beckman HPLC[8] equipped with a Zorbax ODS encapped Sb-C18 column[9], (4.6 by 250 mm, 5 µm particle size). For the ACCase extracts, HPLC solvents were A: water with pH 2.2, and B: acetonitrile. The elution profile was: 60% B, isocratic for 5 min; 60 to 70% B, linear gradient for 10 min; 70 to 100% B, linear gradient for 3 min; 100% B, isocratic for 2 min. The column was then re-equilibrated with 60% B for 5 min before next injection (Tardif and Leroux 1991 with some revisions). The elution was performed at a flow rate of 2 ml $min^{-1}$and an injection volume of 100 µl.

For the ALS extracts, a solvent system of 1% acetic acid in water and methanol at a flow rate of 1.5 ml min^{-1} was followed (Schuster 2007). The elution profile was as follows: step 1, 5 to 20% methanol linear gradient for 10 min; step 2, 20 to 80% methanol linear gradient for 10 min; step 3, 80 to 100% methanol linear gradient for 5 min; and step 4, 100 to 5% methanol linear gradient for 10 min.

Radioactivity for both experiments were measured with an EG&G Berthold[10] scintillation spectroscope. Quizalofop and rimsulfuron standards were included to determine herbicide retention time. Retention time for quizalofop, rimsulfuron, and their metabolites were determined.

Experimental design and data analysis.

The experimental design for all studies was a randomized complete block. Treatments were blocked by harvest time. Treatments were replicated four times, and the experiment was conducted three times in quizalofop and twice in rimsulfurom metabolism studies, respectively. There were no interactions among runs for either study; therefore, data were pooled over runs within herbicide. Data from both studies were analyzed using ANOVA, and means were separated by using Fisher's Protected LSD at $P \leq 0.05$. Metabolism data in the ALS study was subjected to regression analysis using exponential decay models. To determine if differences existed between lines, 95% confidence intervals of the slope were plotted for each genotype in Sigma Plot 10.0[11]. If the lower confidence interval of one equation diverged from the upper interval of another equation then the slopes are deemed significantly different.

Results and Discussion

Quizalofop metabolism. Quizalofop, like other APP herbicides, is applied in relatively inactive form (ester ethyl of quizalofop) that needs to be converted to be biologically active (quizalofop) (Duke and Kenyon 1999). Tardiff and Leroux (1991) reported that aside from the production of active quizalofop, metabolism of quizalofop-ethyl produced another polar metabolite; however, this was not the case in grain sorghum. Active quizalofop was eluted at 2 minutes with quizalofop-ethyl eluting at 10 minutes in the elution profile. Almost 88 and 91% quizalofop-ethyl was metabolized to quizalofop in ACCase-resistant, and -susceptible grain sorghum 3 DAT, respectively. At 5 DAT, remaining inactive quizalofop-ethyl was 6 to 8% in both genotypes and by 7 DAT all inactive quizalofop-ethyl was converted to active quizalofop in all treated plants (data not shown). Other researchers have reported similar quizalofop metabolism rates in other species. Koeppe et al. 1990 observed that ^{14}C residues of quizalofop-ethyl dissipated rapidly in both soybean and cotton plants. There were no differences in quizalofop metabolism in treated grain sorghum plants at any harvest timings indicating that differential rate of metabolism is not a mechanism of resistance in ACCase-resistant grain sorghum.

Rimsulfuron metabolism. Two distinct peaks of radioactivity beside rimsulfuron were observed 3 DAT in resistant and susceptible genotypes (Table 1). The metabolites eluted at 10 and 12 minutes with rimsulfuron eluting from the column at 19 minutes. These two metabolites were present in each treatment but varied according to harvest timings and genotypes although the amount of metabolite that eluted at 12 minutes was generally greater than when eluted at 10 minutes. Based on the mobile phase gradient used, both of the metabolites appear to be hydrophilic. Previous metabolism studies in plants, soil, and water show that rimsulfuron can be rapidly hydrolyzed into metabolite (*N*-(4,6-dimethoxypyrimidin-2-yl)-*N*-(3-(ethylsulfonyl)-2-pyridinylurea)), which itself was transformed into a more stable metabolite (*N*-((3-(ethylsulfonyl)-2-pyridinyl)-4,6-dimethoxy-2-pyrimidineamine)) (Martins et al. 2001; Rosenbom et al, 2010). Koeppe and

Brown (1995) have reported that the metabolism of rimsulfuron in tolerant corn involves hydroxylation of the pyrimidine ring followed by glucosylation. A cleavage of the sulfonylurea bridge also has been suggested.

Initial metabolism of rimsulfuron was rapid in all grain sorghum genotypes but did not increase substantially over time, especially in the susceptible plants. The percentage of the radioactivity recovered from rimsulfuron 3 DAT corresponded to 80 and 83% in the resistant genotypes compared to 87% in the susceptible genotypes (Table 1). At 5 DAT, metabolism was near steady in all sorghum plants but by 7 DAT, resistant genotypes metabolized 4 to 12% more rapidly than the susceptible sorghum. Metabolism rate was similar in both resistant grain sorghum genotypes (TX430R and N223R) in all harvest dates except at 7 DAT (Figure 1). At 7 DAT, TX430R metabolized rimsulfuron 8% faster than N223R. Differences in metabolism were also noted when resistant were compared with the susceptible genotypes (TX430S and N223S). Rimsulfuron declined over time in all treatments.

Differential rimsulfuron metabolism in resistant grain sorghum plants may suggest that sorghum breeders need to incorporate genes that metabolize rimsulfuron in to commercial hybrids to ensure greater rimsulfourn resistance. Differential rimsulfuron metabolism in grain sorghum is consistent with our field observation that grain sorghum recovery from rimsulfuron injury varied among genotypes. These results are not surprising since similar results were reported in corn treated with nicosulfuron (Siminszky et al. 1995).

In the quizalofop metabolism study, results do not support the involvement of differential metabolism in the observed response of grain sorghum to quizalofop.

Metabolism is probably not a mechanism of resistance in ACCase-resistant grain sorghum. This research, however, showed that rimsulfuron metabolism by ALS-resistant sorghum is more rapid than the susceptible genotypes indicating that rapid metabolism is a mechanism that could explain the rapid recovery of grain sorghum plants from rimsulfuron injury observed in the field.

Sources of Materials

[1]Miracle-Gro soluble fertilizer, Scotts Miracle-Gro Products Inc., 1411 Scottslawn Road, Marysville, OH 43041.

[2]Prime Oil, Terra International Inc., P.O. Box 6000, Soiux City, IA 51102-6000.

[3]Tricarb 2100TR Liquid Scintillation Analyzer, Packard Instrument Co., 800 Research Parkway, Meriden, CT 06450.

[4]Whatman International Ltd., Springfield Mill, James Whatman Way, Maidstone, Kent ME14 2LE, United Kingson.

[5]Centrivap, Labconco, 8811 Prospect, Kansas City, MO 64132.

[6]0.2-µm filter, Osmotics Inc., 5951 Clearwater Drive, Minnetonka, MN 55343.

[7]Activate Plus, Agriliance, LLC, P.O. Box 64089, St. Paul, MN 55164-0089.

[8]Beckman high performance liquid chromatograph, Beckman Coulter Inc., Life Science Division, 4300 N. Harbor Boulevard, P.O. Box 3100, Fullerton, CA 92834-3100.

[9]Zorbax ODS endcapped Sb-C18 column, Agilent Technologies, Chemical Analysis Group, 2950 Centerville Road, Wilmington, DE 19808.

[10]Scintillation spectroscope, EG&G Berthold, Postfach 100163, Bad Wilbad D-75312, Germany.

[11]Systat Software, Inc. 501 Canal Blvd, Suite E, Point Richmond, CA 94804-2028.

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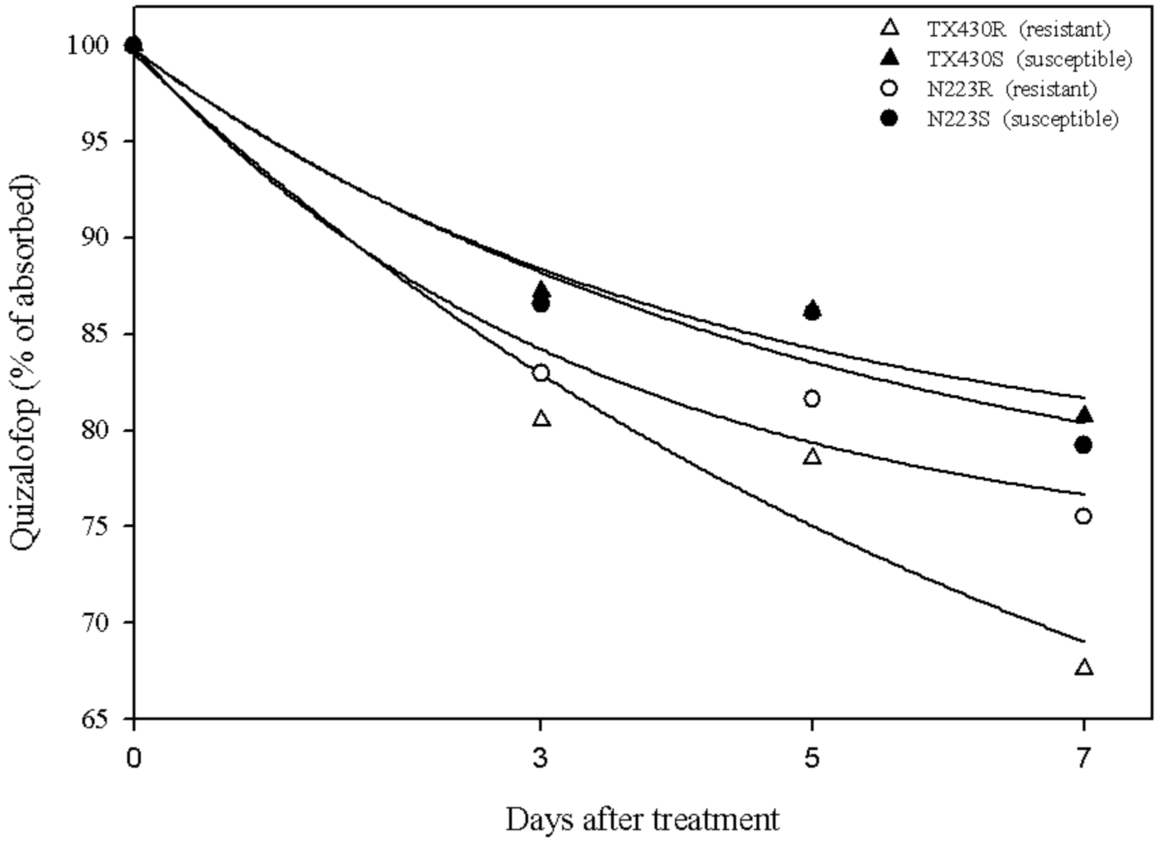
Table 6.1. Rimsulfuron metabolites at 3, 5, and 7 d after treatment in ALS-resistant (TX430R and N223R) and –susceptible (TX430S and N223S) grain sorghum.

Compound	Retention time	TX430R			TX430S			N223R			N223S		
		3 DAT	5 DAT	7 DAT	3 DAT	5 DAT	7 DAT	3 DAT	5 DAT	7 DAT	3 DAT	5 DAT	7 DAT
	min	% of radioactivity											
Metabolite 1	10	7 ± 1	9 ± 1	14 ± 1	5 ± 1	6 ± 1	9 ± 1	6 ± 1	6 ± 1	10 ± 1	6 ± 1	6 ± 1	10 ± 2
Metabolite 2	12	13 ± 1	13 ± 1	13 ± 1	8 ± 1	8 ± 1	11 ± 1	11 ± 1	12 ± 1	15 ± 1	7 ± 1	8 ± 1	11 ± 1
Rimsulfuron	19	80 ± 1	79 ± 1	68 ± 1	87 ± 1	86 ± 1	80 ± 2	83 ± 1	82 ± 2	76 ± 2	87 ± 1	86 ± 2	79 ± 2

Figure 6.1. Metabolism of rimsulfuron in ALS-resistant and -susceptible grain sorghum at 3, 5, 7 d after treatment.



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Appendix B – Differential Response of Grain Sorghum Hybrids to Foliar-Applied Mesotrione

Figure A. Relationship between sorghum grain yield of four sorghum hybrids and visible injury two weeks after treatment at Belleville, Hays, Hesston, and Manhattan.